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DISEASES DUE TO PROTOZOAN PARASITES.

(a) "BLACK-HEAD" IN TURKEYS [AMOEBIASIS (?)]

SMITH (Theobald) & SMILLIE (Ernest W.). **Note on Coccidia in Sparrows and their Assumed Relation to Blackhead in Turkeys.**— *Jl. Experim. Med.* 1917. Mar. 1. Vol. 25. No. 3. pp. 415–420. With 1 table.

The authors were induced to study coccidiosis in sparrows on account of the constant appearance of these birds in the unprotected yards of incubator-hatched turkeys and on account of the view of HADLEY (1910) that the causal parasite of "blackhead" described by the first-named author in 1894 as *Amoeba meleagridis* is merely a stage in the development of the avian coccidium *Eimeria avium*, and is disseminated and transmitted by various species of birds. No experimental proof has, however, been furnished to support the latter view.

In all 54 sparrows were trapped or shot in the experimental turkey yard at Princeton, N.J.; no visible signs of disease were discoverable in any. Search was made in scrapings from the duodenal mucous membrane for the sexual stages and for sporulating forms in the rectal contents by sowing on a medium consisting of 2 per cent. agar in 0·5 per cent. watery salt solution. Sections were also cut from the duodenum for histological study. The results showed that 43 of the birds, or 80 per cent., were infected with coccidia. The sparrows were all adults.

The parasites in the duodenum were all in the sexual-stage, macrogametes and macrogametocytes being the only forms present; they were situated in the nuclear zone of the epithelial cells. The total absence of the vegetative or multiplicative cycle suggests that either the sexual phase is suppressed or else it takes place possibly while the birds are still in the nest.

Cultures of faeces uniformly demonstrated a two-spored oocyst and not the four-spored species described by HADLEY. Each sporocyst contained four sporozoites. According to HADLEY the average measurements of the mature cysts in the turkey were 14 by 21 microns and in the sparrow 22·7 by 21·3 microns, thus indicating that the infesting species are different. The authors' own measurements of the coccidia agreed with those given by HADLEY.

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The authors conclude that the two-spored coccidia found by them belonged to the genus *Diplospora* (Labbé, 1893) or *Isospora* (Sjöbring, 1897), and not to *Eimeria*.

In a footnote the authors refer to a paper recently published by Hadley ascribing the so-called " blackhead " of turkeys to invasions of Trichomonas [see below].

Smith (Theobald). **Some Field Experiments bearing on the Transmission of Blackhead in Turkeys.**—*Jl. Experim. Med.* 1917. Mar. 1. Vol. 25. No. 3. pp. 405–414.

In the experiments described in this paper the author first shows that it is quite possible to breed healthy young turkeys from the eggs of infected adults. Details are given with regard to the precautions taken in incubating the eggs artificially.

It has been often suggested that the parasite of the disease may be carried or disseminated by poultry, but no conclusive experiments have hitherto been made in connection with this point. The author thus exposed two lots of incubator-hatched young turkeys to contact with domestic fowls—one lot to two fowls from a farm where no turkeys were reared and the other lot to two fowls from a farm where blackhead had caused extensive ravages. The results in both cases were negative.

Both lots of young turkeys were subsequently kept in contact with an infected adult turkey on non-infected ground. This exposure led to disease within 17 days. Out of 9 young turkeys 3 died, 2 recovered, while 4 did not show any symptoms.

Exposure to infected adults on infected ground similarly produced the disease in two lots of young clean turkeys. These were exposed on two farms where the disease existed.

Four of the incubator-hatched young turkeys were placed in contact with infected young turkeys. The exposed birds remained healthy although two of the turkeys they were exposed to died of the disease. From previous experiments the author also concludes that infection is either not transmitted at all or only under exceptional conditions by turkeys in the early acute stage of the disease. It is probably carried and shed by those birds which have successfully passed through an attack.

It is then stated that the mode of transmission of the infection is not known ; it appears probable that it is introduced with the food and lodges first in one or both caeca and that faecal matter is the vehicle of infection. The lesions in the intestinal tract are often confined to one caecum only, thus making it improbable that the parasite multiplies higher up. Histological sections of the small intestine moreover failed to reveal any preliminary stage of *Amoeba meleagridis* although in some sections a few rare coccidia were found. It appears that adult turkeys are somewhat more resistant to the disease than the young birds. The lesions in the liver and caeca are associated rather early with an extensive infiltration of round cells while the tendency to necrosis is relatively slight.

BRITISH MUSEUM

HADLEY (Philip B.). **The Part Played by the Goblet Cells in Protozoan Infections of the Intestinal Tract.**—*Jl. Med. Res.* 1917. Mar. Vol. 36. No. 1. pp. 79–86. With 1 plate comprising 11 figs.

In this paper the author indicates the manner in which the flagellate protozoon Trichomonas penetrates the intestinal epithelium in the so-called " blackhead " of turkeys. Organisms of this kind are almost invariably present in the intestinal tract of all animals, but in this disease they appear to undergo a most remarkable development in the deeper mucosa, submucosa, and even in the muscular tissues of the intestinal wall.

The mode of entrance of the parasites was studied by examining the caecum histologically in the early stages of the disease. It was found that the first indication of infection consisted of a bulging out of the lumen of the crypts of Lieberkuhn with a tremendous number of flagellate trophozoites. These had been liberated from the spore mother cells which were abundantly present in the fluid caecal contents and had migrated up the crypts. " They possess the usual Trichomonas features—three anterior flagella and one posterior, a vibratory membrane, nucleus, blepharoplast, rhizoplast, chromatic line, chromatic blocks, axostyle, and nucleus. They are actively motile and measure from 8 to 10μ in length. Their shape is at first crescentic and later oval."

A few of the goblet cells lining the gorged crypts are then seen to contain from one to three or more flagellates. In these cases there is usually a broad opening from the goblet cell into the lumen of the crypt. In many cases there is in addition an opening through the basement end of the cell into a space between the epithelium and its basement membrane and this space may be seen to contain varying numbers of flagellate trophozoites. It is stated that all stages in the passage of the parasite can be seen if one observes a sufficient number of crypts.

The author believes that the parasites effect their passage by means of their " inherent invasive powers " and not on account of the internal pressure within the distended crypts; a goblet cell may be seen to contain only a few parasites although there is considerable distension within the crypt. The invasion of the cells takes place almost entirely in the region of the fundus.

The parasites next accumulate beneath the epithelial cells detaching them from the basement membrane until the greater number of the parasites that were formerly within the crypt become situated beneath the epithelium. This penetration sometimes results in the disorganisation and disintegration of the epithelial cells, but often the pressure now caused by the parasites causes the epithelium of the fundus to be gradually forced out of the crypt space towards the lumen of the caecum.

The parasites meanwhile penetrate the basement membrane and then the remainder of the mucosa is equally overrun until the muscularis mucosae is reached. This retards their progress for a very short time and then they are found developing in the submucosa. At the same time they spread down through the reticular tissue of the cores of the villi until they have reached the tips where they cause the epithelium to separate from the basement membrane and fall

into the lumen of the caecum. Finally, no trace of epithelium can be observed in this area.

The author's observations thus point out that these organisms do not by their own movements effect an entrance between the epithelial cells and that a passage is not necessarily prepared for them by other intestinal organisms such as coccidia or worms. Small numbers of amoebae, together with the flagellates, were sometimes seen by the author in the depth of the crypts, but they were not seen either in the goblet cells or in the deeper tissues; he believes that they are thus of only very slight significance in the pathology of intestinal infections in poultry. "*Amoeba meleagridis* of Theobald Smith is not an exception to that statement, since this organism is not really an amoeba but really the late trophozoite stage of the parasitic flagellate forming the subject of this paper."

Higgins (C. H.). i. **Entero-Hepatitis or Black-Head in Turkeys.**—*Dominion of Canada. Dept. of Agric. Health of Animals Branch.* 1915. Feb. 13. *Bulletin No. 17.* With 4 plates. Ottawa: Govt. Printing Bureau. 1915.

— ii. **Entero-Hepatitis or Black-Head and the Biological Laboratory System of Raising Turkeys.**—*Ibid. Bulletin no. 19.* 1916. Jan. 18.

In these papers the author first notes the ravages caused in recent years throughout Canada, the United States, and elsewhere by the above disease, which was first investigated by Theobald Smith in 1895 and attributed to a parasite called by him *Amoeba meleagridis.* This parasite invades the liver and the two caeca and causes lesions in them, hence the name entero-hepatitis. The losses from the disease are enormous and on some farms amount to 90 per cent. of the young birds. "There is little doubt but that the major portion of the fatalities after the first two weeks result from infection from the parasites of entero-hepatitis or blackhead."

The symptoms of the disease are not particularly characteristic but the affected birds show signs of dulness and exhaustion, and the faeces are more fluid than normal and show yellow streaks and gas bubbles; the head is usually darker than normal. The affected birds usually die in a few days if not treated and on post-mortem examination the liver is found to show yellowish-white circular spots on its surface and usually one or both caeca contain yellowish patches about the size of a walnut; sometimes the whole intestinal tract is inflamed. The author then gives rules with regard to prevention and treatment. Hydrochloric acid has apparently given good results in the treatment of the disease in a few cases.

Infection may be transmitted through adult recovered turkeys, which become chronic carriers of the parasites, and through other poultry such as chickens, which harbour the parasite without being seriously affected by it; ground previously occupied by affected flocks, boots and clothing of attendants, and birds, insects, etc., may carry on the infection. There is a good deal of evidence to show that infection is not transmitted through the egg.

For the prevention of the disease by the so-called "biological laboratory method" of artificial incubation and isolation the following plan was adopted. The section of the farm utilised was divided up

into plots each about 1 acre in surface extent and surrounded by a suitable wire fence. Between the plots a vacant strip from 3 to 5 ft. wide was left and the gateways so arranged that entrance could not be effected direct from one plot to another. The ground containing the turkey plots was surrounded by a boundary fence at a distance of about 10 ft. from the outer plot. Each plot contained a colony house 10 by 12 ft., one side of which is left open. A flock of 25 turkeys could be raised in one plot.

The poults are obtained from eggs by artificial incubation and transferred to the warm chamber or so-called " hover " in the colony house. The author then details at length the care required by these poults. No food is given for the first three days as there is abundant nourishment still in the yolk sac. They are then given a soft mash diet about five times a day and a little water or sour milk and fine grit. In about two weeks time they may be allowed out of the colony house into a run which is covered over so as to protect them against hawks and crows. After a few weeks they are fed three times a day on mixed grain and allowed to roam at will in their plot. Mixed grain together with grit is meanwhile left for them in the colony house and the bedding changed from sand to chaff or straw. Moisture is fatal to young turkeys and every care should be exercised to prevent them getting wet with rain or even dew.

Should one individual poult be found to have a diarrhoea containing yellow particles or froth it must be immediately isolated and the whole of the colony house cleansed and disinfected. The shelters should be thoroughly disinfected at the beginning of each season.

The author considers that the results obtained by this method, as given in the second paper, were quite satisfactory.

(b) Leishmaniasis.

Bouilliez (Marc). **Recherches expérimentales sur** ***Leishmania tropica.*** [Investigations on *Leishmania tropica.*]—***Bull. Soc. Path. Exot.*** **1917. Jan. Vol. 10. No. 1. pp. 66–86. With 1 text fig. & 2 tables.**

The virus used in these experiments was obtained from two natives affected with oriental sore examined at Fort Archambault. A description is given of the parasite and of pathogenicity tests on various experimental animals by means of intradermic and intraperitoneal inoculation. The author's conclusions are as follows:—

" The African monkeys belonging to the family Cercopithecus (*Cynocephalus, Cercopithecus patas, Cercopithecus callitrichus*) are susceptible to *Leishmania tropica*, and in them a cutaneous lesion resembling the human oriental boil is produced at the point of inoculation.

" They do not react to intraperitoneal inoculation.

" The same results are produced in the case of the dog.

" Small rodents belonging to the rat genus, or some nearly related genus, become affected with a generalised infection following intraperitoneal inoculation and this is manifested by the presence of *Leishmania tropica* in the blood-forming organs and certain viscera; this is accompanied generally, after a certain interval, by the appearance of local lesions, during the development of which the infection may disappear from the viscera and organs primarily affected."

LAVERAN (A.). **Au sujet de l'évolution des infections expérimentales des petits Rongeurs par *Leishmania tropica*.** [The Evolution of Experimental Infections by means of *Leishmania tropica* in Small Rodents.]—*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 110–113.

In this article Laveran criticizes BOUILLIEZ's memoir (extracted above). Although the two authors are in agreement on many points their opinions differ as to the sequence of the pathogenic effects produced by *Leishmania tropica* in small rodents. Laveran then details some experiments performed on white mice with the virus in his possession. It is admitted that the virus employed by BOUILLIEZ might have shown greater activity. The author concludes that with the virus of oriental sore which he used in inoculating white mice intraperitoneally or into the peritesticular connective tissue the local effects, ordinarily manifested by the presence of a testicular swelling, precede the general infection; this, moreover, may be altogether absent when an early post-mortem examination is made.

(c) PIROPLASMOSIS.

SPARAPANI. **Trasmissione dell' infezione da *Piroplasma ovis* in tre suini per via digerente.** [Transmission of *P. ovis* to Three Pigs by Ingestion.]—*Pathologica.* 1917. Jan. 15. Vol. 9. No. 196. pp. 21–22.

Three young pigs in a poor jaundiced condition were brought in for slaughter and on post-mortem examination the usual changes accompanying parasitic blood destruction were observed, viz., swelling and softening of the spleen, enlargement and yellowish colour of the liver, congestion of the kidneys, dark red urine in the urinary bladder, and petechiae on the various mucous and serous membranes.

Smears from the peripheral blood stained by Giemsa showed numerous red corpuscles invaded by a piroplasm which from its structure, form, and dimensions was identified as *P. ovis.* It was found on enquiry that a couple of months previously these three pigs had eaten several times the flesh of sheep which had died in their pens. Examination was then made on the farm of blood smears from sheep which were clinically suspected of being affected with piroplasmosis on account of their poor condition and yellow mucous membranes, and in some of these smears numerous corpuscles were found invaded with *P. ovis.*

Though the great majority of observers assert that piroplasmosis is transmitted only by the bites of ticks the author quotes in support of his own observation the results of NAWROTSKY's experiments in infecting dogs by the direct administration of piroplasm-containing blood into their stomachs; also, LANFRANCHI demonstrated the transmission of *T. brucei* and *gambiense* to pups sucking bitches artificially infected.

VAN SACEGHEM (R.). **Cas suspects d'*East Coast fever* au Congo.** [Suspected Cases of East Coast Fever in the Congo.]—*Bull. Soc. Path. Exot.* 1917. Mar. Vol. 10. No. 3. pp. 172–173.

The author observed at Zambi, Lower Congo, in bovines an affection which showed clinical symptoms and lesions corresponding

exactly with those of East Coast fever, but in which he failed to demonstrate the presence of Koch's blue bodies. The symptoms were fever, inappetence, stiffness, progressive paresis of the hind quarters, and enlargement of the glands; there was no haemoglobinuria. In some cases the animals were observed to be quite healthy in the morning, become paralysed in the evening, and die during the night.

On careful examination the possibility of trypanosomiasis, piroplasmosis, anaplasmosis, or poisoning was eliminated.

On post-mortem examination the liver was found to be enlarged and yellowish in colour; the lymphatic glands were enlarged and haemorrhagic; the kidneys and liver were scattered with yellowish-white infarcts, the spleen was not hypertrophied, and the intestine was affected with a haemorrhagic enteritis. In the red blood corpuscles a very few rod-shaped bodies identical in shape with Theileria were discoverable; no Koch's blue bodies were found. The disease terminated in death in all the infected animals and the mortality in the course of a few months rose to 40 per cent. of the cattle of the district.

LEGER (M.) & MOUZELS (P.). **Piroplasme et microfilaire d'un Edenté, le *Bradypus tridactylus* Linn.** [Piroplasm and Microfilaria of an Edentate *Bradypus tridactylus* Linne.]—*Bull. Soc. Path. Exot.* 1917. Mar. Vol. 10. No. 3. pp. 173–176.

In the edentates of French Guiana MESNIL and BRIMONT have described the following haematozoa, viz., an endotrypanum and a trypanosome of *Choloepus didactylus* (two-toed sloth), a trypanosome and a microfilaria of *Tamandua tridactyla*, and a microfilaria of *Bradypus tridactylus*, commonly known as "Ai" (or three-toed sloth).

In the red blood corpuscles of a wild specimen of the last-named species caught by the authors a piroplasm was also discovered. The parasites were fairly numerous and presented different appearances. The most common form was from ·5 to ·8μ in diameter, oval, and almost entirely filled up with an irregularly shaped nucleus staining deeply red. Other parasites were from 1 to 2μ, pear-shaped or oval, and containing a nucleus which was sometimes compact and sometimes represented by a slender filament which assumed very variable shapes. Very rare bacillary forms also were seen, some of which were about 1μ and truly rod-shaped, and others slightly curved, about 2μ in length and match-shaped. The multiplicative forms remained small in size and hardly ever exceeded 2μ in diameter. The chromatin became separated out into from two to six small masses and when segmentation was complete each nucleus was surrounded by a scarcely visible protoplasmic zone.

The red corpuscles invaded were of normal size and appearance while the other corpuscles also presented no changes in morphology or staining-reactions. No anaplasma-like bodies were seen. The leucocyte count was normal.

It was exceptional to find more than one parasite in the same corpuscle and the organism was disposed rather towards the periphery of the corpuscle. Smears from various organs showed no particular abnormality and no bodies similar to Koch's blue bodies were discoverable.

According to the authors the parasite falls into the genus Theileria, created by BETTENCOURT, FRANÇA, and BORGES (1907) for piroplasms of which certain elements are bacillary in form and which in dividing give rise to daughter elements in the form of a cross and very poor in cytoplasm. The authors give the name *Theileria brimonti* to this new species.

A tick belonging to the species *Amblyomma varium* Koch was found on the affected animal.

In smears from the blood and especially from the lung of the above animal a microfilaria was discovered which was identified with *Microfilaria kerandeli* Brimont. The dimensions were as follows :—cephalic spot 4·5μ, from this up to the first clear space 37·5μ, from this up to second clear space 23μ, second clear space 5μ, from this up to posterior extremity 105μ.

(d) SPIROCHAETES.

MACFIE (J. W. Scott). **The Morphology of Certain Spirochaetes of Man and other Animals.**—*Ann. Trop. Med. & Parasit.* 1916. Dec. 16. Vol. 10. No. 3. pp. 305–343. With 6 figs. and 8 tables.

In the introduction to this article the author discusses the measurement of the lengths of spirochaetes as a test of species. It appears to be extremely difficult to distinguish the smaller spirochaetes by morphological characters and in the case of any one species the staining reactions, thickness, and structural characters vary with the chemical composition of the surrounding medium and the method employed in putting them in evidence. The technique of cultivation is moreover laborious and difficult.

Length is perhaps the morphological character which promises to be of greatest assistance in identification, notwithstanding the fact that considerable variations are known to occur in a single species. The method proposed by the author is to draw a large number of spirochaetes with the aid of a camera lucida, to measure the drawings either by the tangent line or compass method, and to plot the lengths as a curve. By this method the great majority of the spirochaetes of the same species are found to measure within a few microns of each other in length.

The measurements may be grouped in the following manner:—

"First there come a few very short forms, which are probably either abnormally small individuals or, in the case of spirochaetes possessing an intracellular phase, immature specimens that have been prematurely freed in the process of making the films. Then there come the normal single spirochaetes which form the dome of the curve and extend over a range of 3 or 4 microns. . . . This is . . . the characteristic length of the organism. Next come the pre-division forms and the forms already differentiated into two daughter spirochaetes, united end to end by a delicate filament; and at this point on the curve a small subsidiary crest or halt in the descent may be observed, due to the overlapping in length of large single and small double forms. Finally there come the abnormally long individuals, including hypertrophic specimens, unusually large dividing forms, incompletely divided organisms, and the multiple forms not infrequently met with in some cases."

The author then proceeds to employ this method in the identification of spirochaetes from the vagina, bladder, and pharynx in cases of human disease. The last part of the article, however, is more especially of veterinary interest and records observations on the intestinal

spirochaetes of a number of the lower animals. Until quite recently, even the intestinal spirochaetes found in man had not been thoroughly studied, and with the exception of a few brief references the author was unable to discover any descriptions of the forms found in domesticated animals. [He appears to be unaware of the recent work of some American authors on the spirochaetes found in the intestinal tract and internal organs in pigs in cases of swine fever.—Ed.]. Our lack of knowledge appears to be due to the fact that hitherto these spirochaetes have been assumed to be non-pathogenic. The spirochaetes found on the surfaces of ulcerated lesions have also been regarded as saprophytic, but in some cases there is evidence that the organisms may spread beyond the superficial lesions and even become blood parasites. In the course of examining blood films from the Accra slaughterhouse in 1914, spirochaetes were occasionally found, viz., in 4 out of 166 cattle, 4 out of 95 sheep, 1 out of 94 pigs, and 1 out of 80 goats. The parasites were of types found to occur in the intestines of these animals and they were always rare in the films. "The intestinal spirochaetes are sometimes present in such enormous numbers that one cannot but suspect that they are not entirely harmless. In man they are often most abundant in cases of diarrhoea, and they are certainly suspected of being directly connected with this condition."

Monkey. Small spirochaetes were found in great numbers in smears made from the large intestine and rectum of a monkey (*Cercopithecus petaurista*) that had died of amoebic dysentery at Accra. When stained by Leishman's method the spirochaetes appeared extremely slender but when stained with gentian violet they appeared rather thicker, about $0{\cdot}2\mu$. The organisms were of the loosely-coiled type. The longest measured 9μ, the shortest 3μ, average $5{\cdot}42\mu$. When plotted the measurements formed a curve similar to that of *S. eurygyrata* —recently shown by FANTHAM to constitute the single species invariably found in human faeces—and it was concluded that this spirochaete of the monkey was morphologically identical with the human species.

Rats. The faeces of a few healthy rats (*Epimys rattus*) were examined and spirochaetes were found to be numerous in some of the animals, scanty in others, but the species of parasite appeared to be the same in each case. The length of the organisms varied from 2μ to 11μ, average $5{\cdot}27\mu$, and it was concluded that they could not be distinguished from *S. eurygyrata* by their morphological characters.

Sheep, cattle, goats, and pigs. A few spirochaetes from the faeces of these animals were measured. The average length was $4{\cdot}7\mu$ in sheep, $4{\cdot}7\mu$ in cattle, $5{\cdot}5\mu$ in goats, and $5{\cdot}2\mu$ in pigs. "Considering the small number of measurements made in each case, the lengths correspond fairly closely with each other and with those of rat spirochaetes and *S. eurygyrata*."

Dogs. In the faeces of an apparently healthy rough-haired terrier of European extraction an exceedingly heavy infection with minute spirochaetes was found. The same organism was also found in two native dogs. There was nothing characteristic about their movements, and structural details were difficult to observe on account of their smallness. Details are given with regard to the shape of the body and coils. The shortest length was 1μ, longest 5μ, average $2{\cdot}7\mu$. The spirochaetes were slender organisms and even the thicker ones were less than $0{\cdot}25\mu$ broad. The ends were tapering and generally

sharply pointed. This spirochaete appears to be a good deal shorter than that described by other authors in dogs, the species described by some resembling *Treponema pallidum*. The spirochaetes described by BALFOUR in the stomach and intestines of dogs dying of experimental trypanosomiasis in the Soudan appear to have been thicker, much more closely and regularly coiled, and longer. The author proposes the name *Spirochaeta canis* for the species described by him.

Cats. The faeces of four cats were examined and in three a very small number of small spirochaetes were found, and these appeared to be identical with those described above as occurring in dogs. In the fourth cat two different spiral organisms were found, the one in the stomach and the other in the large intestine and rectum. The organism found in the stomach was not of the *S. eurygyrata* type. It was a relatively thick organism, so closely coiled that it resembled a screw with a narrow thread; the average breadth was about 0·4 or 0·5μ and the length was from 3 to 8μ, average 5·48μ. This organism was believed to be the same as that found by BALFOUR in the stomach of a dog and referred to above. In the stomach of the same cat a great number of small delicate-looking spirochaetes with pointed extremities, morphologically indistinguishable from *S. eurygyrata*, were found.

The author's conclusions are as follows:—

"1. Spirochaetes of the *S. eurygyrata* type have been found in the faeces of certain of the lower animals examined at Accra.

"2. The first type was found in a monkey, a cat, rats, sheep, cattle, goats, and pigs, and appeared to be morphologically indistinguishable from *S. eurygyrata*, the species found in man.

"3. The second type, for which the name *S. canis* is proposed, was found in dogs and cats. This was a smaller organism, measuring most commonly 2μ to 3μ in length, and about 0·2μ in breadth."

AKATSU (Seinai) & NOGUCHI (Hideyo). **The Drug-Fastness of Spirochaetes to Arsenic, Mercurial, and Iodide Compounds** *in vitro*.—*Jl. Experim. Med.* 1917. Mar. 1. Vol. 25. No. 3. pp. 349–362. With 11 tables.

"It has been known for some time that in trypanosomiasis the trypanosomes which have survived the first effect of an arsenic germicide, such as atoxyl or arsacetin, offer a greater resistance to a subsequent dose of the same drug. By subjecting the organisms to repeated injections of an arsenic medicament, one tends to create, both in animals and in man, a strain or race which resists the arsenic chemotherapy to such an extent that the term arsenic-fastness has come into existence as a brief designation of the modified strain. A similar phenomenon has been observed in spirochetosis in fowls and mammals. Even with a typical bacterium, there seems to exist a possibility of raising the original resistance against a certain arsenic compound to a considerable degree, as was shown by Marks in his experiment with the paratyphoid bacillus, which he was finally able to cultivate in a medium containing eight times the amount of arsenious acid which the organism was able to withstand at the beginning of his experiment. Marks accomplished this within a period of three years by successive transplantations from a weaker to a gradually stronger concentration of the acid."

In this paper the experiments are directed mainly towards studying the effects of these drugs on the spirochaete of human syphilus, *Treponema pallidum*, but the results obtained are, however, of general interest. After enumerating the various methods of studying the sterilising effect of the preparations on this organism the authors state that

the only practicable method is to determine the degrees of tolerance of pure cultures of *T. pallidum* to a given medicament by testing the organisms *in vitro* against gradually increasing doses of the drug. Details are then given with regard to the technique employed in carrying out this method.

The following is a summary of the results obtained.

"In the foregoing experiments we attempted to determine whether or not, by subjecting several varieties of spirochaetes to increasing doses of certain chemotherapeutic agents, a gradual increase of resistance to the latter could be shown. For this purpose, pure cultures of *Treponema pallidum, Treponema microdentium,* and *Spirochaeta refringens* were used against the action of salvarsan, neosalvarsan, bichloride of mercury, and iodine-iodide potassium solution *in vitro.* For culture media, the usual ascites-broth-tissue medium as well as solid ascites-agar-tissue medium was used. After permitting the spirochaetes to grow for a fortnight in media containing certain quantities of each drug, transfers were made from tubes showing various degrees of growth to the next series of tubes containing the same drug in still higher concentrations, and similar transfers repeated every two weeks. The results of the experiments may be briefly summarised as follows:—

"1. *Treponema pallidum* and *Treponema microdentium* have, within three to four months, increased their tolerance to salvarsan and neosalvarsan to five and one-half times their original mark. With *Spirochaeta refringens* the increase was about three times.

"2. Against the action of bichloride of mercury, the amount of increased tolerance of *Treponema pallidum* was about 35 to 70 times the original, while that of *Treponema microdentium* was about 10 times as much and was reached within 10 weeks. *Spirochaeta refringens* resisted 30 times the original dose.

"3. There was an unmistakable increase of resistance of these spirochaetes to the action of the iodine-iodide solution (Lugol's solution) when they were grown for several generations in fluid media containing the iodine solution, but the rate of increase between the initial and the acquired tolerance was slight. In general, the addition of Lugol's solution to fluid media has a weak inhibitory influence upon the growth of the spirochaetes, requiring for the total suppression of growth a quantity of over 0·7 c.c. to 5 c.c. of the culture media. The tolerance reached was for about three times that amount.

"4. A similar tolerance phenomenon has not been established when employing a solid instead of a fluid medium containing the drugs. No explanation is offered except a suggestion that the drugs held in the agar do not enter into combination with certain tissue constituents of the medium as they are able to do with tissue elements in fluid media. This may be a factor necessary for inducing drug tolerance in these organisms *in vitro.*

"5. The increased drug-fastness *in vitro* has a limit beyond which no further advance can be made. This limit varies with different species of spirochaetes.

"6. The acquired drug-fastness *in vitro* gradually disappears when the spirochaetes are cultivated again in the drug-free media for several generations."

KOLMER (W.) & WAGNER (R. J.). **Ueber eine im Magenfundus des Hundes vorkommende saprophytische Spirochäte.** [A Saprophytic Spirochaete found in the Fundus of a Dog's Stomach.]—*Centralbl. f. Bakt.* I. Abt. Orig. 1916. Oct. 12. Vol. 78. No. 5. pp. 383–384. With 1 fig.

Within the gland follicles of the fundus of the stomach of an apparently quite normal dog the authors discovered a very large number of spirochaetes in pure culture, which apparently produced no visible

lesion. Histological examination also showed that there was nothing abnormal in the gland cells. The spirochaete was exclusively extracellular and occurred either singly or in tufts filling up the lumen of the follicles. It measured 10 to 12μ in length by about 0·25μ in breadth and always possessed 8 spirals, each of which was 0·7μ in depth and 1·2μ in length.

Reference is made of the discovery of spirochaetes (*Sp. regaudi*) in the normal stomach mucous membrane of dogs and cats by Regaud (1909). Ball & Roquet (1910), and Lucet (1910), described two kinds of spirochaetes in the stomach of a dog affected with gastro-enteritis, viz., a slender actively motile spirochaete, 5 to 10μ by 0·4μ, and another short thick form 4 to 7μ by 0·8μ. These organisms were not discoverable in the blood. The spirochaete dealt with in this paper is stated to be quite different in appearance from those described by these authors.

(e) Trypanosomiasis.

Velu (H.). **La Trypanosomiase des chevaux au Maroc. (Etude expérimentale.)** [An Experimental Study of the Equine Trypanosomiasis of Morocco.]—*Bull. Soc. Path. Exot.* 1917. Mar. Vol. 10. No. 3. pp. 253–260.

Reference has already been made to the author's observations on the above disease [see this *Bulletin*, 1916, March. Vol. 4. No. 1. p. 10]; its frequency and distinctive clinical characters were described. Sergent, Lhéritier and Belleval (1911) gave the name *Trypanosoma marocanum* to the causal organism [*loc. cit.* 1915. Sept. Vol. 3. No. 3. p. 84].

The author summarises the results obtained by inoculating various animals with blood containing the trypanosome from six different horses. The effects of inoculation and of passage on the various animals are depicted in the form of a genealogical chart.

The following are briefly the author's conclusions.

The trypanosome appeared to be very virulent for the white rat. After an incubation period of about 3 to 4 days this animal showed an acute infection which lasted on an average from 7 to 10 days. On post-mortem examination the spleen was found to be enormously enlarged.

The rabbit appeared to be only slightly affected. The disease took on a chronic form which ran a protracted and irregular course. The symptoms and the lesions were the same as in the case of all other trypanosomiases in this animal.

A considerable number of dogs were inoculated and it was found that the disease in the dog was sub-acute in character with frequent febrile crises. The presence of the trypanosome almost constantly in the blood makes observation easy when dogs are used as experimental animals.

In the case of two mules the course of the disease was relatively rapid as compared with that of the natural affection.

Two goats and two sheep inoculated did not present any trypanosomes subsequently in the peripheral blood nor any symptoms besides attacks of fever and wasting in condition. The two goats recovered,

one at the end of thirteen months and the other at the end of twelve months; the infection could only be placed in evidence by the inoculation of large quantities of blood into dogs.

GREGGIO (G.). **Trypanose des porcs; relations des porcs avec la trypanose humaine dans la vallée de l'Inkissi (Moyen Congo belge).** [Trypanosomiasis of Pigs. Relations between Pig and Human Trypanosomiasis in the Inkissi Valley, Middle Belgian Congo.]—*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 113–117.

In this article the author tabulates the results obtained on examination of smears from the blood of pigs brought to the Kisantu Market from various outlying districts from May to November, 1914. The trypanosome discovered was identified by RODHAIN as *T. congolense*.

The following were briefly the results obtained.

(1) Kisantu district (right bank of the Inkissi). Sixteen affected out of 50 examined, = 32 per cent.

(2) Tumba Mani district (right bank of the Inkissi). Fifteen affected out of 32 examined, = 46·8 per cent.

(3) District to the left of the Inkissi (opposite Kisantu). Five affected out of 12 examined, = 41·6 per cent.

This makes a total of 36 altogether affected out of 94 examined, = 38·3 per cent.

The following are, briefly, some of the deductions drawn by the author from these observations.

The infection has spread almost throughout the whole of the large valley of the Inkissi. The trypanosomes were very few in number in the blood. The trypanosomiasis causes no economic loss as the pigs show no external sign of disease and may be in very good condition.

There is no direct relationship between trypanosomiasis in pigs and in human beings in a certain district, and figures are given showing that the one may be very severe and the other almost entirely absent in a given place. Indirectly, however, serious dangers threaten the districts where pig-breeding has become extensive, on account of the fact that these animals favour the multiplication of Glossina and the most suitable places for rearing pigs are infested with the fly. In the district of Tumba Mani, for example, where pig-breeding has become very extensive, the villages are situated on the tops of hillocks surrounded by pastures consisting of short grass which makes it impossible for *G. palpalis* to propagate. The pigs kept in these villages are now able to find sufficient nourishment for themselves in the valleys and copses alongside the watercourses and are continually moving backwards and forwards to the huts in the villages, bringing along on their bodies a considerable number of *G. palpalis*. The percentage of total deaths due to sleeping sickness among the natives in these villages during the last few years has risen to 42·9 per cent., and the migration of *G. palpalis* on pigs appears to be the only explanation of this increase.

Mention is made of a similar occurrence in the Island of Principe, where, according to the Portuguese Commission on Sleeping Sickness, the role of pigs as carriers of *G. palpalis* was firmly established.

KOLMER (J. A.), SCHAMBERG (J. F.) & RAIZISS (G. D.). **Various Methods for determining the Trypanocidal Activity of Substances *in vitro* and their Relation to the Chemotherapy of Experimental Trypanosomiasis.**—*Jl. Infect. Dis.* 1917. Jan. Vol. 20. No. 1. pp. 10–27. With 15 tables.

—, — & —. **The Numeric Relationship of Infection to the Chemotherapy of Experimental Trypanosomiasis.**—*Ibid.* pp. 35–44. With 7 tables.

In the first of these papers the authors describe a series of rather elaborate methods for determining the toxicity of various chemical compounds on trypanosomes.

The first is called the microscopic method; a series of dilutions of the various compounds are added to blood containing trypanosomes obtained from white rats and the times required to kill the trypanosomes as observed by loss of motility and structural changes are determined by direct microscopic examination.

In the second method, which the authors call the "in-vitro-vivo centrifuge method," the trypanosomes are exposed for a certain interval to a definite dilution of the chemical, are then removed and washed by centrifugation and injected into the peritoneal cavities of white rats to determine whether their destruction has been brought about.

In another method called the "combined in-vitro-vivo method," equal parts of varying dilutions of the chemical under study are mixed with the blood trypanosome emulsion and kept at 37° to 40° C, and then the whole or a part of each is injected intraperitoneally into white rats to determine the degree of trypanocidal activity. As a part of the drug is injected the action of the drug is both in-vitro and in-vivo and due care must be exercised against the administration of lethal doses of the drug.

The most suitable trypanosome for use in the inoculation of rats for these experiments was found to be *T. equiperdum*. The results are displayed in a series of tables; the conclusions are as follows:—

"Trypanocidal tests in vitro have been found of distinct value in chemotherapeutic researches in experimental trypanosomiasis.

"Substances exerting a profound trypanocidal activity in vitro are likely to prove trypanocidal in vivo, provided the drug is sufficiently non-toxic to be administered in adequate dosage.

"With the combined in vitro-vivo method described herein, it has been found possible to detect the trypanocidal activity of new compounds which were without effect in vivo in amounts but slightly less than the sub-lethal dose.

"By the methods described herein arsenobenzol, or salvarsan, has been shown to possess a high trypanocidal activity in vitro.

"In vitro methods have also demonstrated a trypanocidal activity on the part of mercurials which is not apparent in the in-vivo tests."

In the second paper the method of study employed consisted in inoculating rats intra-peritoneally with a certain number (100,000 to 150,000 per rat) of *T. equiperdum* 24 hours before various doses of the drug were administered intravenously. The parasites were not usually found in the peripheral blood until 48 hours after injection or 24 hours after treatment. In this manner the infection was given a 24 hours start and was heavy enough to infect the controls in every instance, yet light enough not to mask finer degrees of therapeutic

effect on the part of the drug under study. The conclusions are as follows :—

"These experiments demonstrated that in the chemotherapy of trypanosomiasis there is an important relationship between the number of trypanosomes injected into the test animal and the trypanocidal activity on the part of the drug.

"This relationship is particularly in evidence with respect to the amount of the drug necessary to effect complete sterilisation ; while rats infected with 500,000 trypanosomes may be sterilised with 0·001 gm. of arsenobenzol per 100 gm. of rat, this is not the case when larger numbers of trypanosomes are injected.

"The influence of numbers is less marked when the rats are very heavily infected, as with numbers over 2,000,000. In these instances, arsenobenzol or salvarsan in dose of 0·001 gm. or 0·6 gm. per 60 kilogrammes of body weight retards the appearance of trypanosomes in the peripheral blood, but does not sterilise. The time of appearance of the parasites in the blood is likewise not greatly influenced by variation in the number used in inoculation.

"The numeric relationship of infection to the results of chemotherapeutic experiments is therefore of considerable importance, and more particularly in comparative tests."

RIECKENBERG (H.). **Eine neue Immunitätsreaktion bei experimenteller Trypanosomen-Infektion : die Blutplättchenprobe.** [A New Immunity Reaction in Experimental Trypanosomiasis: The Blood Platelet Test.]—*Zeitschr. f. Immunitätsforsch. u. Exper. Therap.* 1917. Jan. Vol. 26. No. 1. pp. 53–64. With 3 tables.

The technique employed is as follows :—

Rats are infected with a certain strain of nagana obtained from mice and when the trypanosomes in the blood are fairly numerous the rat is restored to health by the administration of tartar emetic, arsenophenylglycin, trixidin, etc. Three or four days after recovery a drop of blood from this rat is mixed with a drop of 2 per cent. citrate broth on a glass slide, and some trypanosomes of the original strain from a mouse are added quickly and well mixed, covered with a cover glass, and then examined under a high power dry objective.

The author claims that in addition to the usual phenomenon of agglutination one observes in such cases as this in about 15 or 20 minutes that the blood platelets become gradually attached to the trypanosomes, especially on the flagella ; translatory movements are first inhibited and then the trypanosome becomes more and more immobile. A considerable mass of blood platelets is finally seen on the trypanosome when its movements have entirely ceased.

The author was able to show that certain free-lying platelets in the preparation belong to the non-immune mouse.

The serum obtained from the blood of such an immune animal when mixed with the trypanosomes and blood of another animal gave no blood-platelet reaction, but showed the usual agglutination reaction.

This blood platelet reaction was further shown to be different from the agglutination reaction inasmuch as the latter could be produced by the addition of the serum of a normal horse whereas no blood platelet reaction could be obtained in this way. The author then details at length experiments with a number of nagana strains maintained in German laboratories ; tests on guinea-pigs, rats,

and mice showed that the reaction was distinctly specific for each strain and also for each of the recurrent strains.

"In the blood of animals infected with experimental trypanosomiasis and afterwards cured or of animals subject to a chronic laboratory infection, antibodies are found which manifest themselves by the fact that rat or mouse trypanosomes in the citrated blood of these animals become loaded with blood platelets.

"This phenomenon is strictly specific for it only appears if a homologous strain of trypanosomes is employed in the test.

"By this test one can differentiate between the original and the recurrent strain. The reaction appeared in all cases in which an immunity against re-infection occurred."

RITZ (H.). **Ueber Rezidive bei experimenteller Trypanosomiasis. II. Mitteilung.** [Recurrent Infections in Experimental Trypanosomiasis. Second Report.]—*Arch. f. Schiffs- u. Trop.-Hyg.* 1916. Sept. 1. Vol. 20. No. 17. pp. 397–420. With 5 tables.

After discussing the significance of recurrent infections in trypanosomiasis according to various authors, Ritz details the technique employed by him in studying the various strains of trypanosomes produced in the course of recurrent attacks following infection by a single pure strain.

A rabbit was inoculated intravenously with a small quantity of blood from a mouse containing *T. brucei*, PROWAZEK strain, which had been isolated and maintained in mice in a pure state according to OEHLER'S method.

On microscopic examination of this rabbit's blood a few trypanosomes were observed on the first three days after infection, on the 10th to the 12th day inclusive, and again in increasing numbers from the 24th to the 29th day, when the animal died.

Mouse inoculation was performed daily from the rabbit's blood. These gave positive results every day except on the 5th day after infection. The strains obtained on the first four days after infection and again on the nine days following the temporary disappearance of trypanosomes from the blood were kept running separately in mice and employed, together with the original strain used for infecting the rabbit, in a series of reciprocal cross-immunity tests on mice.

The mice used in these tests were infected intraperitoneally with the strain to be examined and then on the third day, when trypanosomes were numerous in the blood, cured by means of an intravenous injection of neosalvarsan. The trypanosomes having then disappeared from the blood the mice were re-infected with the other strains to be tested and the subsequent absence of trypanosomes from the blood or a long-delayed infection indicated immunity, or the identity of the two strains inoculated into the mouse.

In the case of rabbits the author divides the clinical course of infection into three stages, viz., the initial stage, which commences immediately after infection and lasts three or four days, secondly the crisis, during which the trypanosomes may disappear completely from the blood for up to three days as shown by mouse inoculation, and thirdly, the recurrence or the relapse, when the trypanosomes can be demonstrated daily up to death.

By means of the mouse cross-immunity tests it was found that trypanosomes which appeared during the initial stage were identical with each other, and with the original strain.

The results, however, obtained in the tests with recurrent strains are very complicated. The first, third, fourth, fifth, seventh, and ninth day recurrent strains were tested against each other and against the original strain. The results as shown in the tables are briefly as follows :—

Mice immune to the original strain showed no immunity towards any of the recurrent strains.

Mice immune to the first day recurrent strain showed no immunity towards the original or any of the other recurrent strains except that a slight delay in infection was noticeable in the case of re-infection with the third day strain.

Infection with the third-day recurrent strain produced immunity against itself and the first day strain and a slight delay in the case of the fourth day and fifth day strains, and negative results towards the original and the other recurrent strains.

Infection with the fourth day recurrent strain produced immunity against itself and the first day strain and a slight delay in the case of the third, fifth, and seventh day recurrent strains.

Infection with the fifth day recurrent strain produced immunity against itself, the first and the sixth day strains only.

Infection with the seventh day recurrent strain produced immunity against itself and the first day strain and a slight delay in the case of the second day strain.

Infection with the ninth day recurrent strain produced immunity against itself only, and a slight delay in the case of the seventh day strain.

Control mice inoculated with these strains died in all cases on the fourth or fifth day after infection.

The author enters into a long discussion on the significance of these results. His views are briefly that changes take place analogous to those in which "drug-fast" trypanosomes are created. Owing to trypanosome-immune bodies which tend gradually to destroy the strain or strains of trypanosomes present being produced in the serum of the infected animal, "serum-fast" forms are continually appearing and these new strains show different properties to the original strain and to the forms previously formed during the recurrent attack. In the course of a recurrent attack there are thus continually produced in the blood a very large number of new "serum-fast" strains which are probably responsible for the ultimate death of the animal.

Schuscha (A. T.). **Ueber die Wirkung von Emetinum hydrochloricum auf Trypanosomen.** [The Action of Emetine Hydrochloride on Trypanosomes.]—*Cent. f. Bakt.* 1. Abt. Orig. 1917. Mar. 31. Vol. 79. No. 4. pp. 180–183. With 5 tables.

In view of the apparently specific action of emetine in amoebic dysentery, the author performed a few experiments to ascertain whether the drug had the same effect upon trypanosomes. In these experiments mice were inoculated with a strain of *T. equiperdum* which ordinarily produced death in from 7 to 14 days. The toxic dose of emetine was found to be about 0·002 gramme per mouse.

A sub-lethal dose (0·001 gramme) injected on the third day after infection, when trypanosomes first appeared in the blood, was found to have no curative effect, although death occurred in the treated animals two or three days later than in the case of the controls.

Even when injected early, that is, before the appearance of trypanosomes, the drug failed to prevent death. Death, however, was delayed for about ten days when the drug was administered on the day following the inoculation, and approximately the same results were obtained when the drug and the trypanosomes were injected simultaneously.

The author concludes that emetine hydrochloride has only a small protective action on mice after infection subcutaneously with trypanosomiasis (*T. equiperdum*). A curative action, as in the case of amoebic dysentery, does not exist; the action of the drug on the latter disease appears to be quite specific.

SWEZY (Olive). **The Kinetonucleus of Flagellates and the Binuclear Theory of Hartmann.**—*Univ. California Publicat. Zool.* 1916. Mar. 16. Vol. 16. No. 15. pp. 185–240. With 58 text figs.

"Summary.—1. The 'kinetonucleus' is a structure which, in the trypanosomes at least, is not composed of nuclear chromatin, nor can nuclear behavior, shown in mitosis, be claimed for it. It is a structure correlated with an endoparasitic mode of life, and is part of the extranuclear motor apparatus, which, in those cases where its origin can be traced, arises from the blepharoplast, and not by a division of the nucleus.

"2. The 'kinetonucleus' has been developed phylogenetically along two lines: first, from a uniflagellate ancestor possessing a simple basal granule, producing *Trypanosoma, Crithidia* and *Herpetomonas*; second, from a heteromastigote ancestor, also exhibiting only a simple basal granule, along the line of *Prowazekia, Polymastix, Trichomonas*, and the Trichonymphida.

"3. The chromidial body of *Prowazekia* arises as an outgrowth from a simple basal granule. Different stages in its development can be traced from this condition to the 'kinetonucleus' of *P. cruzi*, followed by its backward migration to a position posterior to the nucleus. The various forms which it assumes are all different aspects of the same structure, morphologically and functionally equivalent to the 'kinetonucleus' of the trypanosomes.

"4. The next stage in the development of the 'kinetonucleus' has produced, by a course of parallel evolution along two different lines, organelles in *Polymastix* and *Trypanoplasma* similar in their morphology and behavior in division. These are accessory kinetic structures comparable to the 'kinetonuclei' of the haemoflagellates.

"5. A slight modification of the long, band-like 'kinetonucleus' of *Prowazekia lacertae* is found in the chromatic basal rod of *Trichomonas*. This is an accessory structure intimately related to the blepharoplast and the entire neuro-motor apparatus and probably kinetic in its function, and hence an organelle homologous with the 'kinetonucleus' of the trypanosomes.

"6. A further development of the 'kinetonucleus' is found in the parabasal bodies of the Trichonymphida, a group phylogenetically close to *Trichomonas*. These bodies are accessory kinetic structures and in every respect comparable with the same organelles found in the other flagellates.

"7. This structure is not the kinetic center of the cell, but is an accessory part of the motor apparatus, a kinetic reservoir, hence the term 'kinetonucleus' denotes a wrong interpretation of its function, as does also the name blepharoplast, which is reserved for the definitive basal granule of the flagella plus the centrosome in some cases at least. As a more appropriate substitute for these names, and one which better indicates

its probable relations in the cell, is suggested the term 'parabasal body,' first proposed by Janicki for these organelles in the Trichonymphida. Its application is here considerably extended.

"8. The binuclear theory of Hartmann and the foundation of the order Binucleata, rest upon three main propositions, namely, that the 'kinetonucleus' is composed of nuclear chromatin, that it originates by division of the trophonucleus, and that it divides by mitosis. These facts are contradicted by the results of careful investigations on both the Haemoflagellata and Haemosporidia, where these conditions are claimed to occur, which show, (i) by actual experimental proof, that the 'kinetonucleus' is not composed of nuclear chromatin, (ii) that in no single instance has it been found to arise by division of the nucleus, and (iii) that in no instance has a process of mitosis been found which could be correlated with division of the other organelles and with the cell.

"9. The Haemosporidia are affiliated with the Haemoflagellata neither morphologically nor by a comparison of the developmental data of the two groups. On the contrary, they are more nearly allied to the Coccidia and should be retained therewith in the Sporozoa.

"10. The order Binucleata, being founded on false premises and composed of families totally unrelated either morphologically or phylogenetically, should not be retained as a valid order of the Mastigophora."

(f) Miscellaneous.

Leger (Marcel). **Observations sur quelques Leucocytozoon d'Oiseaux de la région de Reims.** [Observations on Some Leucocytozoa of Birds in the Neighbourhood of Rheims.]—*Bull. Soc. Path. Exot.* 1917. Jan. Vol. 10. No. 1. pp. 28–33.

(1) Leucocytozoon parasites appear to be very common in the blood of crows (*Corvus corax*). Out of 15 birds examined 12 were found affected, for the most part intensely. The infection appears to be very severe in nestlings. The Leucocytozoon is of a rounded type measuring from 12–14μ in diameter and has an oval nucleus which nearly always contains at its thinner extremity a rod-shaped or spherical kinetonucleus. The nucleus of the invaded cell usually forms a crescent embracing in its concavity more than half the haematozoon. The author found that the host cell was a mononuclear leucocyte and was able to prove that the red corpuscles were not invaded, as insisted upon by some authors, by pouring the blood into slightly acidulated water.

Small oval or elongated elements were seen measuring from 1 to 2μ, containing a relatively large red-stained nucleus surrounded by the blue-stained protoplasm. These were richest in the lungs and bone marrow. The next forms, which had grown from 2·5 to 4μ, were found adherent to a depression in the nucleus of the host cell. The growth of the parasite is then very rapid and the nucleus is pushed towards the periphery of the cell but is not disintegrated.

The formation of microgametes was observed when blood had been collected in a solution of potassium oxalate, although this could not be observed in pure blood. The liberated flagella were found to be very active.

In the bone marrow of crows certain forms were observed which seemed to belong to the multiplicative stage of Leucocytozoon.

(2) Leucocytozoon were also frequently found in the common magpies of the Champagne district (*Pica melanoleuca*). The parasite was identified with *L. berestneffi* Sambon, 1908, described by Sakaroff in Transcaucasian magpies, 1893.

(3) A Leucocytozoon parasite was also found in a screech owl (*Asio accipitrinus*), and the author believes that this parasite is identical with *L. ziemanni* Laveran, 1902.

YAKIMOFF (W. L.) & SAPHRONOWITSCH (R. A.). **Parasites du sang des animaux en Transcaucasie.** [Blood Parasites of Animals in the Transcaucasus.]—*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 98–100.

(1). Grahamella in rodents of the Caucasus.—This protozoon was found in the blood of a hamster (*Cricetus phoca*) and in a species of field mouse. In stained specimens the parasites appear as rod-shaped organisms measuring from 0·75 to 1·5μ, variable in number, but sometimes very numerous, and staining a violet-blue colour. No visible ill-effects were produced on the hosts. Anaplasms were also observed in the field mouse. The name *G. ninae kohl-yakimovi* is given to the parasite of the hamster.

(2). Theileria in the field mouse.—The blood of several of these animals in the district of Kars was examined and in all presented distinct polychromatophilia, but no poikilocytosis or normalblasts. In one case the authors discovered small rounded intra-corpuscular elements each consisting of protoplasm staining blue and a peripheral nucleus occupying nearly half the parasite. No pear-shaped or rod-shaped elements were observed. These bodies are identical with similar forms found previously by Yakimoff. The name *Theileria rossica* is given to these elements.

(3). Leucocytogregarina of a fish.—Gregarines have recently been frequently discovered in the leucocytes of mammals, but none have hitherto been described in the leucocytes of fishes although they have been found on several occasions in the red corpuscles. In the fish examined the gregarine was found inside a large mononuclear; it measured 9μ by 4·5μ and possessed one rounded and one elongated pointed extremity. The name *Leucocytogregarina ninae kohl-yakimovi* is given to this parasite.

LEGER (M.) & MOUZELS (P.). *Plasmodium* **de** *Iguana nudicollis.*—*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 95–98.

The small lizards of the species *Iguana nudicollis* Cuvier found in Guiana are often observed to harbour a Plasmodium which invades the red blood corpuscles without producing in them any deformity or change in the staining reactions. A single corpuscle may contain two or three parasites.

Asexual elements.—The youngest form, which is also the form most commonly found, consists of a minute body scarcely 1μ in diameter made up almost entirely of a nucleus containing a brightly staining karyosome towards its periphery. The growing stage presents a variety of appearances—ring-shaped, sometimes pear-shaped, dumb-bell-shaped, rod-shaped, etc.—and often containing in addition to the nucleus a very dense coarse chromatin particle staining garnet-red. The adult form becomes amoeboid or lozenge-shaped and never exceeds 5μ in diameter. The nucleus usually disappears and the chromatin forms a diffuse network in the protoplasm and later becomes

divided into three or four small masses placed towards the periphery of the parasite. The above forms rarely show any pigment granules. Segmentation of the schizonts never takes place in the circulating blood but can be observed in the organs, especially in the lung. These dividing forms are found outside the corpuscles, and are regularly rounded in appearance, measure from 5 up to 7μ, and contain an abundance of dark brown pigment. Schizogony thus takes place with the formation of four merozoites.

Sexual elements.—The gametes are fairly numerous in smears from the organs especially from the spleen but are extremely rare in the peripheral blood. They are always outside the corpuscles. Macrogametes are rounded or oval, from 3·5 to 6μ in diameter with a centrally placed chromatin mass and contain a very abundant black irregular mass of pigment. The microgametocytes are rather smaller in size and the nucleus is made up of loose lightly staining chromatin; the pigment is made up of greenish-brown short rods.

A form believed to be a female element undergoing parthenogenesis is also described. No periodicity was observed in the schizogonic cycle when the blood was examined for several days. Reference is made to other species of plasmodium found in lizards by Carini, Wenyon, and other observers, but the parasite described in this paper is said to bear a close resemblance to *Pl. praecox* found on the West Coast of Africa and *Pyrhemocyton tarentolae* Chatton & Blanc. The name *Pl. carinii* is proposed for this new species.

Yakimoff (W. L.). *Prowazekia ninae kohl-yakimovi—Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. p. 101.

This protozoon was discovered in infusions of hay from various parts of Russia. It possessed one principal nucleus, a blepharoplast, and an anterior and a posterior flagellum, each with one or more basal granules. The parasite was cultivable on Frosch's and Musgrave and Clegg's agar.

Yakimoff (W. L.) & Schokhor (N. J.). *Leucocytogregarina musculi* **A. Porter à Pétrograde.**—*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 100–101.

A leucocytogregarine was discovered by Porter (1908) in mice in London and later by Sangiorgi (1912) in Turin.

This parasite was found by the author in smears from the organs of a mouse at Petrograd, and he believes it to be identical with the organism described by Porter and Sangiorgi.

DISEASES DUE TO METAZOAN PARASITES.

Yakimoff (W. L.) & Collaborators. **Microfilaires des animaux au Turkestan russe.** [Microfilariae of Animals in Russian Turkestan.] —*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 102–105.

Reference has already been made to microfilariae described by the authors in horses, asses, mules (*Microfilaria ninae kohlyakimovi*), and

camels (*Mf. camelensis*). [See this *Bulletin*, 1916. June. Vol. 4. No. 2. p. 69.] In this article microfilariae are described in the following species of animals.

(1). *Dogs.* The blood of 597 dogs from various districts was examined and 61 were found infected (10·21 per cent). The monthly infection was as follows :—March, 4 per cent, April, 4·1 per cent., and May, 14·1 per cent. The parasite measured from 245·44 to 333·70μ in length, by 7·16 to 8·52μ in breadth. It had the same appearance as microfilariae in other animals except that the sheath could not be stained. It usually contained five clear spots. A description is given of the internal structure of the parasites and differential blood counts of the host. The microfilaria is stated to be identical with *Mf. immitis*. Sometimes adult filariae were found in the heart and large blood vessels. The intestinal contents of Hippoboscidae caught on the dogs disclosed microfilariae.

(2). *Bovines.* Examination of the peripheral blood of 316 animals at the slaughterhouse failed to disclose any microfilariae. The affected seat was, however, later found to be the liver. Examination of the peripheral blood of a further series of 1,019 animals revealed filarial embryos in two cases. In the livers of 510 animals examined the parasites were found in 43 cases (8·4 per cent.). The sheath of the parasite stained very well. It contained 3 clear spaces. Its measurements, including the sheath, were on an average 250μ in length by 8·25μ in breadth. Sometimes smaller parasites were found resembling the sausage-like stage of *Filaria bancrofti* in the thoracic muscles of *Culex fatigans* (Low). The parasite is believed to be identical with the embryo of *Filaria labiato-papillosa*.

No microfilariae were found in the peripheral blood of 1,173 sheep and 671 ewes, and in 106 sheeps' livers.

(3). *Frogs.* Small microfilariae were found in the blood of *Rana viridis*. The sheath was well developed and stained red. Including the sheath they measured on an average 46μ by 4·24μ. They contained closely packed nuclei.

Leger (André). **Microfilaires d'oiseaux du Sénégal.** [Microfilariae of Birds in Senegal.]—*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 106–109.

After reviewing the list of microfilariae already found in the blood of many species of birds in several parts of the world, especially in Africa, the author describes three new species discovered by him in the following birds in Senegal. The adult forms of the embryos were not discoverable.

(1). The Giant Heron (*Ardea goliath* Temminck).—The microfilariae found in this bird were surrounded with a compact sheath, measured on an average 220 by 3·5μ, contained several distinct nuclei, and possessed active wriggling but only slight translatory movements. The caudal extremity was hook-shaped and the posterior end blunted. The parasite showed no periodicity, and it apparently had no ill effect upon the health of the host.

(2). Snipe (*Gallinago nigripennis* Linné).—The microfilaria was very rare in the blood. It measured 130 by 14μ, contained distinct

cellular nuclei, was surrounded by a compact sheath showing no striation of the cuticle, and the posterior extremity was elongated and slender; it presented only slow creeping movements.

(3). The Large White-Throat (*Crateropus platycercus* Swainson).—In one bird examined an extremely severe infection was found (from 7 to 10 per field of the microscope). Morphologically these were found to belong to two different species.

The first was very closely ensheathed, measured 250 by 5·5μ, and presented very little activity, its movements being rather leech-like; it contained packed distinct nuclei, its posterior extremity was bulbous and the central clear spot was very apparent.

The second microfilaria was much smaller in size (80 by 5·5μ) and much less numerous in the blood (1 to 25 or 30 of the former). It was not ensheathed and it exhibited extremely rapid translatory movements. The cellular nuclei were indistinct, there was no striation of the cuticle, and the posterior extremity was blunted.

CLELAND (J. B.), DODD (S.) & FERGUSON (E. W.). **Further Investigations into the Etiology of Worm Nests in Cattle due to** *Onchocerca gibsoni*. **No. 2.**—*Commonwealth of Australia Publication.* 41 pp. Melbourne: **Albert J. Mullet, Govt. Printer.**

The investigations forming the subject of this communication are a continuation of those previously carried out in the State of New South Wales; details of previous experiments carried out by Cleland in conjunction with various authors have already been published in Government Reports commencing in July, 1913. The experimental work was carried out at Milson Island where conditions appeared favourable for work on account of the natural infestation with worm nests of the herd of cattle kept on the Island. From previous experiments carried out in this locality it seemed very clearly indicated that the vector was a flying insect, and that it probably was either *Stomoxys calcitrans* or one or more species of mosquito or of tabanids. The preponderance of evidence seemed to be in favour of Stomoxys and special attention was thus directed to this insect.

Three fly-proof cages for the accommodation of susceptible clean calves were erected and full details are given with regard to these structures.

Into one of these cages were placed two calves following each other at about three months interval; large numbers (about 11,000) of Stomoxys that had fed on infected cattle or on worm nests from the abattoir were introduced at short intervals over a period of three and six months respectively for the first and second calf. Notwithstanding the feeding of such large numbers of Stomoxys, of which some at least would be infected with Onchocerca larvae, it was found on slaughter that neither of these calves contained worm nests.

A similar experiment was simultaneously carried out in order to ascertain the role of mosquitos as possible vectors. Large numbers (over 2,000) of mosquitoes nearly all belonging to the species *Culicelsa vigilax*, and a few *Scutomyia notoscripta*, *Culicelsa annulirostris*, and *Stegomyia atripes*, were fed first on infected material as above and then introduced at various short intervals into the fly-proof cage. No worm nests were discoverable in these calves on post-mortem examination.

In the adjoining fly-proof cage one control calf was kept during these experiments and a few tabanids caught on infected cattle introduced. No worm nests were subsequently discoverable on slaughter.

Another control calf was kept in a pen where it was exposed to the attacks of flies and also to direct contact with the infected cattle. After nearly a year this calf was killed and on careful search a small worm nest was detected near the sternum, under the oblique muscles over the abdomen. A detailed account of the flies prevalent at various times during this exposure are given.

A further series of miscellaneous experiments with various diptera were performed.

Experiments to test the life of the larvae from worm nests placed in cow dung showed that they were not capable of surviving for 24 hours. Owing to the short life of the larva outside the body and also in view of the fact that no permanent natural water existed on the island it seems improbable that a water or moisture dwelling in vertebrate is the intermediate host.

Transmission by the inbibition of secretions could only be effected by some species of Musca which are in fact extremely numerous on cattle in Australia. The life of the larval worm is, however, extremely short in these insects and no larvae have been found round the eyes, nostrils, and mouths of cattle where these flies commonly settle. In connection with the possible transmission by biting insects or other invertebrates the incidence and description of a long series of flies are enumerated.

The source through which *Onchocerca gibsoni* was introduced into Australia has previously been discussed by Cleland and Harvey Johnston, who suggested that it had been introduced with buffaloes into the Northern Territory of Australia, and Gilruth and Sweet, who considered that it had been introduced by means of ordinary cattle from Java or some other of the Malay Islands. The authors now state that " a further consideration of the sources from which cattle were imported into Australia in the early days of settlement, together with greater detail as to the distribution of worm nests in cattle in New South Wales, has made us reconsider both these possibilities and we have now come to the conclusion that it is by no means improbable that *Onchocerca gibsoni* reached Australia amongst the early importation of cattle from India into the neighbourhood of Sydney and from this source spread northwards with the distribution of cattle."

With regard to the probable vector the authors conclude that it is unlikely to be Stomoxys or mosquitoes but that it is probably some tabanid ; the distribution of onchocerciasis seems to correspond with the prevalence of tabanids. Details regarding infested cattle on Milson Island conclude the paper.

van Saceghem. **Dermatose et gale démodectique des bovidés.** [Dermatitis and Demodectic Mange of Bovines.]—*Bull. Soc. Path. Exot.* 1917. Feb. Vol. 10. No. 2. pp. 117–120.

The dermatitis which occurs among bovines in the tropics and is caused by *Dermatophilus congolensis* is often confused with bovine demodectic mange. These two skin affections are, however, easily distinguishable bacteriologically and clinically.

Demodex folliculorum var. *bovis* is characterised by the length of its rostrum and cephalothorax, which is almost equal to that of the abdomen. It was first described by GROS (1845), by FAXON (1878) in America, and GRIMM and OEHL in Germany.

The lesions produced by the demodex in Europe are mild, but in South America on the other hand losses are caused on account of the diminution in value of the hide.

GRIFFITHS (1915) describes severe lesions caused by the Demodex in bovines in Nyasaland.

A large number of cases of demodectic mange were observed by the author in the Belgian Congo both among European and native breeds as well as in Indian hump-backed cattle. The disease is characterised by the following clinical symptoms:—

(1) The presence of comedos from the size of pins' heads up to hens' eggs containing brownish matter, and no crusts; (2) the chronic and slowly progressive character of the disease; (3) no effect on the general condition; (4) young animals not affected; (5) occurrence in the dry season as well as in the rainy season in the tropics.

The spread of the disease was prevented by regular dipping in arsenical baths (sodium arsenite, paraffin, soap, and water).

Dermatophilus congolensis, the cause of tropical dermatitis in bovines, is a filamentous bacterium and appears in two forms (1) straight or curved filaments, sometimes branched and containing fine granules, (2) isolated cocci.

The disease is identified clinically by the following characters (1) the formation of crusts, the hairs over which are erect; (2) rapid spread over the animal; (3) complications which may bring about death; (4) young animals and adults affected to the same degree: (5) a seasonal affection which is only seen in the acute form during the rainy season. The specific treatment for this disease consists in the application of an ointment made of 5 per cent. carbolic acid in vaseline.

The two diseases may exist at the same time on one animal.

HADWEN (S.) & BRUCE (E. A.). **Anaphylaxis in Cattle and Sheep, produced by the Larvae of *Hypoderma bovis*, *H. lineatum* and *Oestrus ovis*.—*Jl. Amer. Vet. Med. Assoc.* 1917. Apr. Vol. 4. No. 1. pp. 15–44. With 15 figs.**

The results of numerous interesting experiments described in this paper are briefly summarised by the authors as follows:—

"Anaphylaxis has been reproduced in cattle, sheep, and small animals, with extracts of the larval forms of *H. lineatum*, *H. bovis*, and *Oestrus ovis*.

"The 'acute and chronic' forms have been reproduced, coinciding with Richet's definition.

"The reactions can be induced by crushing and returning an extract of an animal's own larvae into the jugular, showing that larvae living in the animals make them receptive.

"Natural cases of anaphylaxis are described where no injection had been given, and where injury had ruptured the larvae subcutaneously, liberating their contents in sufficient quantity to produce shock.

"A paper by Ries is reviewed in which the author ascribed the cause of infectious anaemia as being due to *Gastrophilus* sp.: it seems probable that he was dealing with reactions such as we describe.

"Animals which had recovered from the reaction were found to be immune for varying periods.

"The symptoms in 'acute' anaphylaxis were immediate; the first noticeable sign being an extremely tired look, succeeded almost immediately by salivation, tears and defaecation, then by signs of asphyxia, and death. In the 'chronic' form the symptoms were a little less rapid and not so severe, in addition there were edemas especially of the eyelids and anus, and marked irritation of the skin.

"Small animals were sensitized with warble extracts and showed signs of anaphylaxis following the second injection.

"Eye and other local reactions were obtained with extracts applied to the mucous membranes. In cattle the reaction was specific for extracts of *Hypoderma*, and in a horse for *Gastrophilus*.

"It would appear probable that similar reactions will be obtained in other animals with their own parasites."

RODHAIN (J.) & BEQUAERT (J.). **Matériaux pour une Etude Monographique de Diptères parasites de l'Afrique. Deuxième partie. Révision des Oestrinae du Continent Africain.**—*Bull. Sci. France et Belgique.* 1916. Nov. 25. Vol. 50. Nos. 1–2. pp. 53–165. With 29 figs. & 1 plate. [Reprinted from *Rev. App. Entom.* 1917. Apr. Vol. 5. Ser. B. Pt. 4. pp. 49–50.]

"This paper deals with the sub-family Oestrinae, which, in the authors' opinion, does not correspond entirely with the intestinal *Oestridae* of Brauer. They exclude the genus *Aulacephala*, Macq., of which the larval stages are unknown and which appears to be more closely allied to *Trixa*, B. B., as well as *Cephenomyia*, Latr., and *Pharyngomyia*, Schin., which they place, as does Girschner, in the Calliphorinae. The chief characters of the larva at the third instar, the pupa and the imago of the Oestrinae are given. On these characters this sub-family would include only the genera *Oestrus*, L., *Cephalopsis*, Towns., *Rhinoestrus*, Br., *Kirkioestrus*, R. and B., and *Gedoelstia*, R. and B. The authors consider a classification based on the characteristics of the larvae can be only a provisional one and is justified only where the adult insect is unknown. These genera form a homogeneous group and might reasonably be treated as sub-genera of a single genus *Oestrus*, L. The distribution of the spines in the larvae at the third instar are not considered to have more than a minor value in classification, as they are probably very variable in different individuals at the same stage in any given species.

"The larvae of the Oestrinae are parasitic in the sinuses of the bones of the skull of various wild and domestic mammals and have been met with accidentally in man. In the latter case they never attain their full development and, when present, are usually localised in the eyes, in which they cause conjunctivitis. Since the cavities of the skull communicate with each other and also with the throat, these larvae may occur in the pharynx and larynx, and have even been found in sheep in the bifurcation of the trachea down to the large bronchial tubes. This often occurs after the death of the host, but probably never does so in the living animal. In antelopes several larvae of different species are usually found at the same time. The larvae of *Oestrus*, *Gedoelstia*, and *Kirkioestrus* are found only among the hollow-horned ruminants, those of *Rhinoestrus* among the equines, pigs and hippopotamus, while those of *Cephalopsis* are peculiar to the camels. Information as to the different species and their respective hosts is given in detail in a table.

"The mode of nutrition of these larvae is not well known, but it is to be supposed that they feed on the mucus of the passages in which they are found. The importance of breeding them is emphasised, though hitherto all attempts to rear them to the adult stage in captivity have failed. The adult flies will not feed or pair in captivity.

"An account is given of the organism, *Herpetomonas oestrorum*, which parasitises these larvae. Six larvae of *Rhinoestrus nivarleti*, R. and B., taken from the same individual of *Potamochoerus porcus*, L., were all parasitised by a flagellate closely resembling and perhaps identical with this.

"A key to the genera of the Oestrinae is given and descriptions with discussions on synonymy, hosts, and geographical distribution of the following species:—*Cephalopsus titillator*, Clarke, *Oestrus ovis*, L., *O. aureoargentatus*, R. and B., and *O. variolosus*, Lw., *O. macdonaldi*, Ged., *Rhinoestrus nivarleti*, R. and B., *R. hippopotami*, Grünb., *R. purpureus*, Brauer, *R. phacochoeri*, R. and B., *Gedoelstia cristata*, R. and B., *Kirkioestrus surcoufi*, Ged., *K. blanchardi* and *K. minutus*, R. and B.

"A bibliography of 46 works is given."

Piot (J. B.). Maladie des Tiques. Traitement préventif et curatif pour l'Egypte.—*Bull. Union Agriculteurs d'Egypte.* 1916. Nov.-Dec. Vol. 14. No. 117. pp. 85–88. [Reprinted from *Rev. App. Entom.* 1917. Apr. Vol. 5. Ser. B. Pt. 4. pp. 51–52.]

"Cattle in Egypt, if left untended, frequently become entirely covered with ticks. It has been proved that infection in the tick is hereditary and that the piroplasm occurs in the salivary glands of the larvae, which can therefore directly inoculate the host. Piroplasmosis in cattle may be acute or chronic, the acute form being often responsible for a mortality as high as 80 per cent. The only curative treatment which the author has found to give complete satisfaction, other than quinine and trypanblue, is arrhénal; a hypodermic injection of one dose of one gramme is generally sufficient to allay the fever. This treatment to be effectual must be given as soon as the symptoms of the disease appear. Preventive measures should be directed against cattle sheds as well as against the cattle themselves. The earthen flooring should be frequently renewed and all cracks and crevices which could form a shelter for the female tick during oviposition should be filled up. A coat of quicklime should be given occasionally to the wall supporting the manger. Each animal should be thoroughly curry-combed, which is a far more effective method of removing all kinds of parasites than the use of any drug. By these simple means, the Administration have succeeded in reducing the cases of disease among their cattle to a minimum."

Fletcher (T. Bainbrigge). Report of the Imperial Pathological Entomologist.—*Rept. Agric. Res. Inst. & Coll. Pusa, 1915–1916.* Calcutta. 1916. pp. 78–84. [Reprinted from *Rev. App. Entom.* 1917. Mar. Vol. 5. Ser. B. Pt. 3. pp. 38–39.]

"Observations have been continued on the life-histories of various Tabanidae occurring at Pusa. Notes are given on the life-histories of *Tabanus nemocallosus*, the larvae of which are not, apparently, cannibals; *T. albimedius*, which is parasitised by a small Chalcidid, and has three broods annually; *T. striatus*, which also has three broods; *T. sanguineus*; *T. hilaris*; *Chrysops stimulans*, of which the larvae feed on dead earthworms and are probably also cannibals; the larvae of *Gastroxides aler* were found in hollows in tree-trunks.

"An account is given of transmission experiments with biting flies in the zone where surra is prevalent. *Tabanus albimedius* and *T. striatus* were found capable of transmitting the surra organism, not only by an interrupted feeding, but also by complete feeding. *T. albimedius* was proved to transmit surra 24 hours after feeding on an infected animal, but it failed to transmit disease after a longer interval. *T. striatus*, however, was found capable of transmitting surra (in one experiment with two flies) as long as 72 hours after feeding on an infected host. Smaller species, such as a *Tabanus* near *virgo*, were tried, but were not found to transmit disease. *Ctenocephalus felis* and dog and cattle fleas were also found incapable of transmitting surra. *Philaematomyia insignis* is only capable of transmitting surra in the case of an immediate interrupted feeding, the maximum interval, between leaving an infected host and commencing to feed on a previously uninfected animal, for which positive results were obtained, being only seven minutes, although

positive results were obtained by direct inoculation of abdominal contents of infected flies as long as 28 hours after feeding on a surra-infected animal.

"Surra is not transmitted hereditarily to the progeny of infected females of *Tabanus striatus*, *T. albimedius*, or *Philaematomyia insignis*. . . ."

"Many maggots causing myiasis in domestic animals have been received and the flies bred out from them."

"Among the ticks received, *Ornithodoros savignyi* was under observation throughout the year. A species identical with, or near, *O. lahorensis* was received from Agra. Both were fed on goats."

Bouet (G.) & Roubaud (E.). **Répartition des Glossines à la Côte d'Ivoire.** [Distribution of Glossina in the Ivory Coast.]—*Bull. Soc. Path. Exot.* 1917. Jan. Vol. 10. No. 1. pp. 37–39.

In a former publication Bouet (1907) gave a few indications on this subject but he now admits that he was mistaken in identifying *G. morsitans* in the Ivory Coast. The true *morsitans* does not appear to exist in this colony and the species was in reality the nearly related *G. longipalpis*. The following is the distribution of the various species in the light of the authors' present knowledge.

From the coast up to latitude 6° N. *G. palpalis* and Glossina of the *fusca* group predominate. Between 5° 30′ and 5° 50′ is the only part of the fly belt where *G. pallicera* can be found and this part also contains almost all the *medicorum* and *nigrofusca* types identifiable in the colony.

Towards 6° 20′ a belt containing *G. longipalpis* commences and this extends and becomes absolutely predominant throughout the hinterland of the Ivory Coast up to the tenth degree, forming in connection with similar belts of the Gold Coast and of Togoland a large invaded tract between the coast and the Sudanese region. Towards the eighth degree *G. tachinoides* appears and predominates across the northern part of the colony.

Working northwards from the coast there are thus four principal Glossina belts, viz., a *palpalis* and *fusca* belt, a *pallicera* belt, a *longipalpis* belt, and a *tachinoides* belt.

Jack (Rupert W.). **Tsetse Fly Investigations, Sebungwe, August-September, 1916.**—*Report to Director of Agriculture, Salisbury, Rhodesia. British South Africa Company.* Dated 23rd September 1916. [MS. Report received in Colonial Office, November 27, 1916.]

The author journeyed in a south-westerly direction from Salisbury towards the Gwai River observing the extension of the area infested with tsetse especially along the water-courses traversed. It was found that the fly had spread rapidly in certain parts of the district, especially at the south-western corner of the big fly belt where the fly has advanced in a southerly direction from the Mzola to the Kana River, and in a westerly direction to the Shangani River, i.e., along the two confluent water-courses running into a stream which runs in a north-westerly direction into the Zambesi. The area around the confluence of these streams is not yet infested. The author believes that tsetse was carried down southwards from the upper

stream, which is dried up in the dry season, to the lower one by means of large bands of game moving down to the latter stream in the evening to drink. A further spread southwards towards the Gwai River is anticipated. A large increase in the number of fly is reported at the head waters of the Mzola River, and extensions of the infested areas are noted in the neighbourhood of the Sengwe and Sasame Rivers.

In the course of observations on Meare's Farm (Sikombella River), evidence was obtained in support of YORKE's theory that trypanosomes of the *T. pecorum* type are capable of being transmitted from an infected to a healthy ox by some agency apart from tsetse fly. The oxen which had survived after visiting a known fly belt in October died in December following their return, and in the meantime transmitted the disease to twelve other oxen on the farm. In March five oxen again journeyed over a distance of 80 miles to another fly belt and on their return over 20 cattle contracted the disease on the farm. No tsetse fly could be found on the farm and the fly has not hitherto been discovered within a radius of 30 miles of the farm.

Experiments upon the effect of the exclusion of game upon trypanosomiasis are suggested and for this purpose the necessary arrangements are enumerated that would have to be made at Sipani Vlei, " an area which from its limited extent, intense infestation, and isolated position in the dry season, is admirably suited for an experiment."

ROBERTSON (Muriel). **Report upon the Present Conditions of the Siroko Valley, Mount Elgon.** Dated 21st January 1914.—19 pp. With 1 map. [MS. Colonial Office Report.]

The area investigated as to the distribution of *Glossina palpalis* from 17th November to 31st December, 1913 consisted of a number of valleys and water courses taking their origin from the eastern slopes of a short rugged ridge of mountains, the highest point of which is Mount Elgon, situated on the border between the Uganda Protectorate and British East Africa. The main stream—the Siroko River—takes its origin from the south-eastern slopes of the mountain, then runs in a northerly direction for a distance of over 30 miles, and becomes lost in marshy swamps. This river and two other small rivers which run together eastwards into it a few miles from its source are fringed with irregular, short, narrow strips of forest in which moderate numbers of tsetse were discovered; the fly was not however found at the upper parts of these rivers although the vegetation in many places appeared well adapted to harbour tsetse. No tsetse was found on the banks of the rivers originating to the north of the last-named rivers, nor in the marshes into which they emptied themselves. The main valley is continuous to the west with the great Kumi plain and is isolated from other fly areas. The distribution of the fly in the valley is indicated on the accompanying map.

1,004 flies were fed on two healthy dogs and one healthy goat with negative results. Dissections of the flies subsequently showed the presence of flagellates in the gut of 24; flagellates were also found in the proboscis of one of these but they were not attached and were few in number. These organisms were of the *T. grayi* type and " this type of flagellate forms no part of the life-cycle of any of the pathogenic

group of mammalian trypanosomes;" they were most probably derived from the crocodiles which are very numerous in all these valleys. Nine inoculations were made into healthy dogs, of the blood of buck shot in the valley with negative results.

No case of sleeping sickness or of cattle trypanosomiasis has yet been reported in the valley and the author's observations indicate that "the mammalian group of trypanosomes has not yet arrived in the Siroko Valley fly-belts."

The valley is at present administered as a closed area in so far as the cultivation of the land and the building of permanent huts is concerned, but there is no attempt to exclude people entering the valley for hunting, fishing, etc.

The author points out that "an area opened under proper regulation achieves a more satisfactory level of safety than a similar area inefficiently closed." Moreover in this case the extent of the area to be cleared is not excessive nor is the clearing of a very difficult type. Measures are then suggested for repopulating the district. The most economical and efficient method of dealing with the conditions consists in "the temporary imposition of restrictions of intercourse and the gradual but complete clearing of the area by the population settled." There are already in existence schemes for the control of plague and of rinderpest and these might be adapted respectively to prevent the introduction of human and animal trypanosomiases.

The land in this district is for the most part very fertile and is much needed to relieve the congested population of other districts.

In the second part of the report Miss Robertson details points concerning the safe opening and administration of the district.

MYCOTIC DISEASES.

Epizootic lymphangitis.

Velu (H.). La lymphangite épizootique; symptomatologie, d'après 300 observations personnelles. [Epizootic Lymphangitis; Symptomatology according to 300 Personal Observations.]—*Bull. Soc. Cent. Med. Vet.* 1917. Mar. 1. *Rec. de Med. Vet.* 1917. Mar. 30. Vol. 92. No. 6. pp. 99–104.

"Epizootic lymphangitis is at present considered to be a disease of the lymphatics and mucous membranes, characterised always and exclusively by the presence of buds and cording of the lymphatics." This, however, does not correspond with the author's experience and he thus classifies the disease into two varieties, viz., (1) an atypical lymphangitis which remains localised in the inoculation wound, and (2) a typical lymphangitis which is manifested by cutaneous lesions (cording and buds) and sometimes by ulcers on the mucous membranes.

Atypical lymphangitis. The most striking characters of the lesions are, resistance to cicatrisation and the formation of fistulae without apparent cause. Very often the original fistula formed becomes constricted and small buds appear around it which later discharge

and give rise to new fistulae often communicating with the original one. One finds on examination of these fistulae that they terminate in serious lesions—necrosis of bone, ligaments, or connective tissue—which account for their persistence and the abundant suppuration. The nature of this variety of the disease has hitherto been overlooked by practitioners as, owing to the favourable results following surgical operation, the lesions were supposed to be due to ordinary causes. Microscopic examination, however, invariably reveals the true nature of the disease and enables one to employ a specific treatment.

The various forms of this atypical lymphangitis observed by the author were as follows:—(a) simple shallow ulcers, of frequent occurrence, and (b) simple fistulous wounds, both occurring as complications of ordinary wounds; (c) a testicular lesion, as observed by TEPPAZ in the course of typical lymphangitis, was observed by the author in five cases of this atypical kind; (d) osseous lesions, observed 17 times; (e) conjunctival ulcers, observed in six cases, bi-lateral, and resembling summer sores in appearance; (f) cryptococcic phlebitis (one case); (g) cutaneous form (two cases) resembling contagious pustular dermatitis in appearance; these cases were characterised by the appearance of a small number of buds disseminated over the body and limbs and not united by visible lymphatic vessels.

Typical lymphangitis. In this form the resistance of the tissues apparently becomes broken down and invasion of the lymphatic vessels takes place from the original wound after an interval which may vary from a fortnight to six months according to the author's observations. This form is generally cutaneous, but it often affects the mucous membranes as a complication, following generalisation, or auto-inoculation.

The cutaneous form may be observed anywhere on the body and the course of the disease is always the same. From the original cicatrised or uncicatrised wound very prominent cords are seen along the lymphatic vessels making their way towards the nearest superficial lymphatic gland. Small intradermic swellings of variable size appear along their course giving them a beaded appearance. These swellings become extremely sensitive and the pain often causes great alterations in the temperament of the animal. The swellings after a very variable interval become converted into small abscesses and burst, the smallest discharging a greyish-red liquid which is sticky and can be easily spread out, and the largest abscesses discharging a laudable pus which is of a creamy, thick, mucous appearance, is not sticky and is spread out with difficulty. The very large deep-seated abscesses give rise to a less homogeneous, more serous-looking, pus.

After puncture the pus changes in character and each bud becomes an irregularly shaped protruding ulcer showing no tendency to cicatrisation and oozing a clear liquid from its surface. The inflammation of the lymphatic vessels disappears eventually leaving only the ulcerated buds and sometimes complications in the lymphatic glands, which may attain a considerable size, suppurate and become perforated with fistulae, discharging a pus extremely rich in cryptococci.

Lesions on the mucous membranes.—These most often appear as final complications in cases of generalised lymphangitis and according

to the seats affected they assume very varied characters. On the lips and on the external wings of the nostril one finds small papules which may be mistaken for horse pox papules. After a few days these papules become transformed into pustules and ulcerate, small raised umbilicated ulcers remaining which discharge a colourless oily liquid. From the internal canthus of the eye a chain of small ulcers was observed in some cases to run up and down the head giving an appearance very similar to that of summer sores. On the conjunctiva, pituitary mucous membrane, and borders of the lips are sometimes seen ulcers which may, at first sight, be mistaken for those of acute glanders. The ulcer, however, is different in that its base protrudes and forms a prominence from 1 to 2 mm. in thickness on the mucous membrane. The ulcer is surrounded by a lightly coloured zone and it shows no tendency to cicatrisation. The ulcers sometimes become confluent and then give rise to a dull-white granulating mass. This condition leads to a painful swelling, and sometimes suppuration, of the submaxillary gland.

Boquet (A.) & Nègre (L.). **Sur la culture du parasite de la lymphangite épizootique.** [Cultivation of the Causal Organism of Epizootic Lymphangitis.]—*Bull. Soc. Path. Exot.* 1917. Apr. Vol. 10. No. 4. pp. 274–276.

In preceding communications the authors claimed to have observed the development of the above organism in mycelial form in the water of condensation of various media. The medium which they now state to be the most favourable for growth consists of a filtered decoction of dried horse dung in water (400 grammes per 2 litres) containing 1 per cent. peptone, 1·8 per cent. agar, and 4 per cent. glucose. When pus taken from an unopened abscess is sown on this medium and incubated at 24° to 26° C. the cryptococci are found after 18 to 24 hours to increase in size and become rounded and granular. After 48 hours the rounded forms are filled with oily drops and throw out filaments which become segmented and attain a length of from 75 to 100μ. Although there was no visible growth the organism grew more rapidly and much more abundantly than on the other media formerly tried. Sub-cultures were, however, not obtainable on this medium and the growth of the mycelium in the original tube stopped after from 15 to 20 days.

It appeared that these failures might be due to the absence of pus in the sub-cultures and in order to make the conditions analogous to those on the original medium the authors smeared the surface of the horse-dung agar with a sterilised maceration of the lymphatic glands of a horse. Six weeks after inoculation a few very small colonies were observed, which increased slowly in size during the following fortnight both in the case of a primary culture and a first sub-culture. The colonies were spherical, projecting, wrinkled, and greyish, and became more and more adherent to the surface of the medium; the largest was about the size of a pin's head. The colonies were found to be composed of a tangled mass of mycelial filaments, granular in appearance, and often deformed by chlamydospores. At the extremities and on the walls of these filaments large rounded or oval cells were formed by a process of budding. The bud gradually

became more and more constricted at its base and was then attached by a short narrow stalk to the mother cell before it finally became detached. These free double-walled cells then contained three or four elements identical with the cryptococci found in pus. These elements were so rare that the authors could not identify them by the usual staining methods, but morphologically they presented all the characters of the asci of ascospores.

The authors conclude that if these results are verified the causal organism of epizootic lymphangitis belongs to the genus Endomyces.

BACTERIAL DISEASE.

MASON (F. E.). **Tuberculosis in Camels.**—*Jl. Comp. Path. & Therap.* 1917. Mar. Vol. 30. Pt. 1. pp. 80–84.

"Tuberculosis appears to be very rare in camels living under normal or natural conditions in all countries except Egypt." Isolated cases have been recorded by LINGARD (1905) and LEESE (1908) in India, by ARCHIBALD (1910) in the Soudan, and also in Algeria.

In Egypt, however, since the first case was reported by LITTLEWOOD (1888), numerous cases have been recorded and the proportion of camels found affected at the Cairo abattoir, where during recent years up to about 2,000 camels are on an average slaughtered yearly for human food, varied from 1·63 to 5·4 per cent. in various years. The higher percentages have been noted since the war, when Egyptian camels only were slaughtered and no Syrian and Arabian camels, which previously were never found affected, were brought in.

The characters of the bacillus on culture and inoculation into small animals indicated that it is invariably of the bovine type. The source of the disease in camels can be easily traced to the custom of the fellaheen of keeping camels in close association with cattle. "All the evidence collected within the last six years tends to show that tuberculosis is not *par excellence* a disease of camels, but that infection takes place primarily from cattle."

The usual method of infection appears to be undoubtedly by inhalation and the lungs are invariably infected. Under ordinary conditions when diet is liberal and work moderate the disease runs a very chronic but progressive course. Under adverse conditions such as continuous transport work in the desert, rapid emaciation sets in with continuous fever, a profuse glairy discharge from the nostrils, pleurisy or peritonitis, and a firm swelling of the superficial lymphatic glands.

On post-mortem examination one finds that the lungs and bronchial glands are invariably infected; "grapes" are frequently seen on the pleura; the parietal pleura frequently escapes infection; the lesions in the lung may take the form of discrete miliary tubercles or a whole lobe may become consolidated and filled with caseous matter and fibrous tissue. There is a marked tendency towards the formation of fibrous tissue. The disease not infrequently produces pericarditis and peritonitis while lesions are not uncommonly

also found in the liver, spleen, kidneys, and on the mucous surface of the trachea. Generalisation takes place in about 7 per cent of cases.

The lesions show the usual histology of tuberculous lesions with the exception that giant cells are rare. Tubercle bacilli are fairly easily discoverable. Calcification in a fine granular form sets in early.

The tuberculin test may be successfully employed. In connection with this test the author gives the following figures concerning the normal temperature of healthy camels at rest:—

"Extreme variation between early morning and late afternoon, from 35° C. (95° F.) to 38·6° C. (101·6° F.).

"At 6 a.m., 35° C. (95° F.) to 37·5° C. (99·6° F.); usual temperature 36·2° C. (97° F.).

"At 6 p.m., 37·4° C. (99·2° F.) to 38·6° C. (101·6° F.).

"A temperature over 37·5° C. (99·6° F.) at 9 a.m. is abnormal.

"A temperature over 38·7° C. (102° F.) at 6 p.m. is abnormal."

DISEASES DUE TO FILTERABLE VIRUSES.

(a) Rabies.

Konrádi (Daniel). **Die Vererbung der Immunitat gegen Lyssa. Dritte Mitteilung.** [Heredity of Rabies Immunity. Third Report.]—*Cent. f. Bakt.* 1. Abt. Orig. 1917. Jan. 30. Vol. 79. No. 2. pp. 80–82.

—. **Die Vererbung der Wut. Vierte Mitteilung.** [Heredity of Rabies. Fourth Report.]—*Ibid.* pp. 82–84.

In a former series of seven investigations Konradi demonstrated that immunity towards rabies was transmissible from the mother to the foetus [see this *Bulletin*, Vol. 2, No. 2, p. 94, and Vol. 4, No. 2, p. 62] and that this hereditary immunity is manifested only by the first generation and lasted ten months or longer.

Experiment 8. A bitch was immunised (3–5–1912) by means of a virus which had been submitted to a series of passages through a frog and through fifteen guinea-pigs successively; 2 grammes of the brain of the last guinea-pig were emulsified and injected under the skin of the bitch. The bitch subsequently showed no symptoms, but a guinea-pig inoculated sub-durally with this emulsion died in 52 days of typical rabies.

One hundred and ninety-five days (14–11–1912) after the inoculation the bitch gave birth to six pups, two of which remained alive.

One of the pups when nearly a year old (8–11–1913), was inoculated into the muscles of the back alongside the spinal cord with a dose of 1 c.c. of "street" virus. The dog remained alive and showed no symptoms of disease for one year. However, control animals, one dog and one guinea-pig, died after 26 days, and a rabbit inoculated sub-durally died after 20 days, of rabies.

The second pup was inoculated in the same way at the age of 2 years (13–11–1914) with the virus of human rabies. Twenty-four days afterwards (7–12–1914) it died of typical rabies. The mother's

immunity was of still shorter duration as the bitch died subsequent to a test inoculation performed ten months (11–3–1913) after immunisation.

Experiment 9. A bitch was immunised (13–2–1914) with a virus which had passed successively through a series of eighteen rabbits, then through a fowl, and finally through a guinea-pig. The fowl died in 59 days after sub-dural inoculation and the guinea-pig in 94 days. The bitch was injected subcutaneously with 2 grammes of cord and subsequently showed no symptoms, but two control guinea-pigs injected sub-durally died in 20 and 22 days, respectively, of typical rabies.

In order to confer a still greater immunity the bitch was again inoculated (16–4–1914) with 2 grammes of cord containing virus which had successively passed through a fowl and through four guinea-pigs. This inoculation was also well stood, but a control guinea-pig died in 20 days of rabies.

The bitch had a litter of three pups (30–7–1914) of which two remained alive for observation. At the age of 1 year 8 months and 25 days (632 days) (24–4–1916), the two pups were simultaneously inoculated into the muscles of the back with "street" virus. Six months later they were still alive and well, but control animals inoculated in the same manner, viz., one rabbit and one dog, died after 20 and 39 days, respectively, of typical rabies.

The mother in this experiment also showed a shorter immunity, for it died of rabies after an earlier inoculation (11–12–1914).

The immunity of the two pups thus lasted for at least 632 days, which is much longer than EHRLICH and his successors accepted as a certain proof.

Experiment 10. A bitch was inoculated subcutaneously with an emulsion made from 24 grammes of cord containing virus obtained after 57 successive passages. The virus was kept for five days in carbol-glycerine with a view to attenuation, but when tested on a guinea-pig and a rabbit no weakening was found in reality to have taken place. On the 160th day after inoculation (4–4–1915) the bitch whelped a litter of four pups. These four pups died at the age of 14, 17, 30, and 34 days, respectively, all showing Negri bodies in the central nervous system and, in the case of the last two, typical symptoms of rabies.

Experiment 11. A bitch was injected (12–1–1915) subcutaneously with 4 grammes of guinea-pig cord containing virus which had passed first through a fowl and then through a series of eleven guinea-pigs.

Three days afterwards the mother gave birth to four pups. All four pups died, when 3, 15, 16, and 16 days old, respectively. Negri bodies were discoverable in all except the second pup that died, and in each case a guinea-pig inoculated into the muscles of the neck from the cord of a pup died of typical rabies in from 14 to 19 days.

In this case it was thus shown that the rabies virus appears as early as three days in the blood of the mother.

It is added that the mother had obtained no immunity for after a test inoculation on 7–4–1915 she died within 12 days of typical rabies.

Experiment 12. A bitch was immunised (31–1–1915) by subcutaneous inoculation with 3·5 gramme guinea-pig cord, the "street" virus in this case having been passed first through a fowl and then through a series of twelve guinea-pigs.

Forty-nine days after immunisation the mother aborted a well-developed foetus from which a guinea-pig was inoculated into the muscles of the neck. This guinea-pig died 15 days afterwards of typical rabies. The mother was inoculated twice (5–5–1915, and 15–1–1916) subcutaneously with an emulsion of guinea-pig cord as above for further immunisation and was still alive in October, 1916.

The author concludes that "from investigations made in this direction in the course of the last twelve years it is an established fact that the rabies virus is transmitted from the mother to the foetus."

(b) Rinderpest.

Shilston (A. W.). **Rinderpest. Preparation of Anti-Serum.**—*Agric. Res. Inst. Pusa. Bull. No. 64.* 1916. 18 pp. With 21 tables. 1916. Calcutta. Supt. Govt. Printing. India.

In this paper a series of experiments is summarily described performed with the object of raising the potency and effecting economies in the manufacture of anti-rinderpest serum at the Muktesar laboratory.

A comparative study of the yields resulting from different methods of separating serum from the blood has already been described in this *Bulletin* [see Vol. 4, No. 4, p. 188. Norris, R.V.] and the enormous quantities issued have also been referred to [see Vol. 5, No. 1, pp. 66 and 68].

Reference is made to the literature dealing with this subject. It appears that no accurate data have hitherto been published, and in the light of recent experiments in the production of other anti-sera it also appears that the interval between the last injection with virus and the first bleeding has been unnecessarily long. As the result of a series of observations made by Holmes the most recent method employed at the laboratory consisted in bleeding at 15 and 17 days' intervals after injection, at the rate of 6 and 8 cc. per pound body weight. The method investigated by the author consisted in bleeding each time at the rate of 6 cc. per pound of body weight at 8, 12 and 16 days after the injection of virus.

In the manufacture of anti-serum both hill bulls and plains buffaloes are employed. The latter animals are much less susceptible to rinderpest than hill bulls, but they were introduced by Holmes for the manufacture of serum as the supply of hill bulls is strictly limited. Hill bulls are, however, necessary for the production of virulent blood for inoculation and for routine serum testing.

In the first experiment 10 hyper-immune serum-making hill bulls were selected and divided into two lots; after inoculation with virulent blood these were bled at the two different series of intervals described above. The yields of serum per pound body weight were noted at each bleeding for each animal. The mixed sera obtained at each bleeding were then tested on susceptible hill bulls which were simultaneously inoculated with 0·5 cc. of virulent blood. In each test six animals were employed and were divided into three lots, the doses injected in the successive lots being 27, 54 and 81 cc. of serum per 600 lb. body weight. In addition two control animals were inoculated with virulent blood only in each test, and bled to death for virulent blood at the height of the attack.

From these tests it was found that:—

"(1) The rinderpest antibodies were present in the serum of hyper-immune animals in full amount eight days after the injection of virulent blood.

"(2) At the second bleeding on the 12th day the antibodies were still present in sufficient quantity for 27 cc. per 600 lb. body weight to protect hill bulls against the injection of virulent blood, which is equal to 1·5 cc. per 600 lb. in the case of plains animals.

"(3) The serum from a third bleeding four days later was still protective in doses of 54 cc. for hill bulls or 3 cc. for plains bulls per 600 lb. body weight.

"(4) The serum from a first bleeding 15 days after the injection of virulent blood was fully protective in doses of 27 cc. for hill bulls or 1·5 cc. for plains bulls, but at a second bleeding on the seventeenth day the potency had fallen so that 54 cc. per 600 lb. body weight were required to give full protection to hill bulls."

In the second experiment the value of the mixed serum obtained from three bleedings on the eighth, twelfth and sixteenth day after injection was compared with that of mixed serum from two bleedings taken on the fifteenth and seventeenth days. The procedure was the same as before. It was found that both lots of serum were protective in doses of 27 cc. for hill bulls or 1·5 cc. for plains animals per 600 lb. body weight, but that the "three bleedings" serum gave a distinctly better test.

In the third experiment the animals bled at the two series of intervals described above were inoculated with rinderpest virus at an interval of 12 days from the last bleeding; the two lots of animals were then bled as before and the mixed serum from each lot was compared. It was found that neither of the mixed sera in this experiment gave as good results as the sera of the second experiment. "The difference was, however, not sufficiently marked to draw any definite conclusions as to the greater production of anti-bodies having occurred in the animals previously bled three times than in those only bled twice."

In the fourth experiment it was decided to compare the "three bleedings" and "two bleedings" systems on a large scale, testing the two methods on hill bulls and buffaloes separately. 64 hill bulls and 18 buffaloes were thus employed in the first system and 103 hill bulls and 58 buffaloes in the second system. The yields of serum per pound body weight were noted as before and the mixed sera from each bleeding of hill bulls and of buffaloes were tested on susceptible hill bulls. The conclusions were as follows:—

"(1) The mixed sera obtained by both the 3-bleedings and 2-bleedings methods from hill bulls and buffaloes were protective in doses of 27 cc. for hill bulls or 1·5 cc. for plains animals per 600 lb. body weight; this is less than one-third of the standard dose issued.

"(2) The mixed sera obtained from hill bulls by the two methods were of about equal value according to the tests.

"(3) The mixed serum obtained from buffaloes by the 3-bleedings method was markedly stronger than that obtained by the 2-bleedings method and would probably protect in even smaller doses than 1·5 cc. per 600 lb. body weight in plains animals."

The author next gives a summary of the records of the body weights of the animals, the amounts of blood taken and yields of serum at each bleeding in experiments 2 and 3. A comparison of the figures

for the "three bleedings" and "two bleedings" method in this observation showed :—

"(1) An increased yield of serum after each injection of 2·81 cc. per lb. body weight by the former method (9·6 cc.) as against the latter (6·79 cc.); this means a total increased yield of 41·4 per cent.

"(2) An increased percentage yield of serum from the blood taken of 5·3 per cent. by three bleedings (54·5 per cent.) over that from two bleedings (49·2 per cent.), the proportion of serum increasing with each successive bleeding of a series."

These results were confirmed by the yields of serum obtained in the experiment conducted on a large scale (Experiment 4).

[It appears that the author draws rather far-reaching conclusions from the results of titration experiments in which a comparatively small number of animals were used. The very small dose ($1\frac{1}{2}$ cc. per 600 lb. body weight) of serum calculated to be sufficient for immunising plains animals rather inclines one to think that a large proportion of these animals must possess a high degree of natural immunity towards rinderpest and that thus the small proportion of losses in an outbreak dealt with would not be altogether attributable to the use of the serum; this statement does not, however, minimise the value of the experiments performed with the object the author had in view.—Ed.]

von Ostertag (R.). **Ueber Rinderpest. Ein Beitrag zum Stande und zur Bekämpfung der Tierseuchen in Deutsch-Ostafrika.** [Rinderpest. A Contribution on the Incidence and Eradication of Animal Diseases in German East Africa.]— *Zeitschr. f. Infektionsk. parasit. Krankh. u. Hyg. der Haustiere.* 1916. Sept. Vol. 18. No. 1. pp. 1–48. (Continued from Vol. 17).*

(4) *Symptoms and post-mortem appearances.*—Under this heading, which covers half the present paper, Ostertag details at great length the appearances presented in rinderpest-affected animals in outbreaks in various districts. These were furnished in official reports in 1912 and 1913 by the Government Veterinary Surgeons (Manleitner, Lichtenheld, Münchgesang and Wölfel), and they are also largely the result of the author's own observations. Although they contain nothing really new, the conclusions arrived at from this careful study are reproduced as follows :—

"The results obtained by me show how varied might be the clinical picture in different animals of the rinderpest raging at that time in German East Africa, and confirm the truth expressed by one of the first workers on rinderpest, Lorinser, that 'the totality of the symptoms appertaining to rinderpest can never be established in a single affected animal and scarcely in an affected herd, but is always more complete the greater the number of sick animals examined'; Gerlach further adds that neither in one affected animal nor in one affected herd, but only in several herds of different breeds in different outbreaks, countries, and seasons, can the totality of the symptoms and the variations in the progress of the disease be investigated. However, as symptoms of the disease in living animals which must always create a suspicion of rinderpest in one animal my observations point out the following :—

"The simultaneous existence of lachrymation and diarrhoea with a high degree of fever at the commencement and a normal or subnormal temperature towards the end of the disease. The suspicion is

* The publication containing the first part of this article has not yet been received, but an extract of it will appear when it is obtainable.

strengthened by the appearance of diphtheritic patches and ulcers on the mucous membrane of the mouth and of reddish streaks on the mucous membrane of the posterior extremity of the alimentary tract. In dead or slaughtered animals the suspicion of rinderpest is aroused by the presence of croupous deposits on, or a diphtheritic inflammation of, the mucous membrane of the mouth and of the fourth stomach, of the solitary follicles or Peyer's patches, or other parts of the intestinal mucous membrane; reddish streaks on the rectum or colon, enlargement of the gall bladder, and a thickened condition of the gall, and all these changes without any specific lesions of the spleen and of the kidneys.

"The changes of the gall bladder and gall appear both in rinderpest in German East Africa as well as in European rinderpest—which was designated centuries ago as 'Great Gall' (Grossgalle) owing to the most striking lesion in the gall bladder—to be the most constant sign of the disease. All other changes may vary but one can nevertheless by sufficient observation of the mucous membranes of the digestive tract in one or other locality (mouth, fourth stomach, small or large intestine) discover one or more of the specific lesions of rinderpest; here one must have in mind that in East Coast fever similar ulcers may be found on the mucous membrane of the fourth stomach as in the case of rinderpest.

"The results of post-mortem examination will thus, taken in conjunction with the symptoms observed during life (lachrymation, diarrhoea, and at the beginning a high morning temperature), regularly enable one to make a certain diagnosis even in a single case, the correctness of which can be confirmed without any doubt by the further course of the disease in an outbreak. A negative blood examination gives further support in a single case, but a positive result (showing piroplasms, trypanosomes) on the other hand gives no ground to exclude the possibility of the disease, for the piroplasm and trypanosome infection may be an accidental complication. This latter statement must be laid stress on for on the strength of a positive result obtained by the examination of one blood film forwarded for examination wrong action might be taken if one overlooked the possibility of the existence of rinderpest and if one neglected the further examination of the affected herd."

(5) *Transmission of rinderpest to other domesticated animals, especially sheep and goats.*—In the European outbreaks which occurred over 60 years ago great losses were observable amongst sheep and goats which had been in contact with rinderpest-affected cattle. Great losses also occurred among them in Egypt at that period. GERLACH, however (1865), denied that serious losses could be caused in sheep and goats although a fever and slight symptoms could be set up by means of artificial inoculation. In German East Africa no disease appeared in sheep and goats in the course of the epizootic studied by the author and according to the natives none died in the outbreak of rinderpest which occurred in 1892, and this circumstance saved the Masai from starvation after their cattle had been destroyed.

Reference is also made to the experiments of KOCH on the inoculability of the virus to sheep and goats and to his suggested use of the sheep as a medium for transporting rinderpest virus. Ostertag, however, states that the facts established by KOCH in 1896 with regard to South African sheep and goats did not apply to German East African sheep, as the rinderpest with which KOCH experimented was of a different character from that of German East Africa. Experiments carried out by Ostertag's assistants gave negative results as far as visible symptoms were concerned.

The solution of the question as to whether East African sheep or goats could be used for eliminating piroplasms infective for bovines

from the rinderpest virus would be of great practical importance and moreover blood obtained from rinderpest-affected cattle loses its virulence in a comparatively short time (50 hours) and thus cannot be carried *in vitro* over long distances. Sheep and goats are in addition more easily transported and run less risk of spreading the infection during transit.

Tests made in Mpapua showed that the rinderpest virus could be carried in sheep and goats without weakening the virus and this made it possible to obtain a reliable virus for inoculation when severely affected cattle from which virus could be obtained were not available.

(6) *Eradication of rinderpest.*—Immediately on the receipt of reports from MANLEITNER and WÖLFEL that rinderpest had broken out the Government of German East Africa took steps to suppress the disease. The infected districts were isolated and the export of samli (animal fat) and hides was only allowed on condition that the samli was heated to at least 60° C. and the skins were thoroughly dried in the sun. Twelve thousand doses of serum from Cairo were at hand and a further 15,000 doses were obtained from Kabete, British East Africa. All proved effective. Two Institutes for the manufacture of anti-rinderpest serum were set up, one at Engare-Nanyuki and another at Mpapua.

The establishment at Engare-Nanyuki erected by WÖLFEL is described at some length. This station was placed in a forest and native reserve on the east side of the Meru Mountains about 5,000 ft. high, and about 2,500 acres in extent. It was bounded on the east by the deep Engare-Nanyuki River and on the other sides it was surrounded by forest and mountains in which leopards and hyaenas abounded. The spread of infection by the breaking out of infected animals was thus prevented on all sides. In the neighbourhood of the station the game was killed off and the station itself was divided by ditches into different parts, the northern ones being used as pasture and quarantine land. Cattle on arrival were passed successively through two kraals. In the first the blood was examined microscopically and the unaffected animals were then driven to the second kraal and washed with Cooper's dip. They were then put into the quarantine enclosure. A description is then given of the laboratory installations and animal sheds, etc. Seventy-two serum cattle were kept at the place during Ostertag's stay.

The method of immunisation was as follows:—Two strains of rinderpest virus were employed. An initial immunity was produced by means of the ordinary double inoculation, the dose of serum being small so as to obtain a powerful reaction. After four weeks' rest the animals each received 1,000 cc. of virulent blood and after a further two weeks 3,000 cc., injected subcutaneously over the whole body in quantities of 100 cc. After the disappearance of the reaction—about 14 days after the second hyper-immunisation—the first blood extraction period commenced and this consisted of six extractions, each of $3\frac{1}{2}$ to $4\frac{1}{2}$ litres according to the size of the animal, taken at intervals of four days. The animals were then given three weeks' rest and again inoculated with 3,000 cc. of virulent blood to be ready again in another three weeks time for a further blood extraction. This was repeated until the animals were no longer fit for serum production.

The virus was obtained from young heifers and calves which had been inoculated with from 5 to 10 cc. of virulent blood. The animals showed a rise of temperature in within three to five days after the infection and after seven to eight days the first symptoms of disease appeared. By means of blood and gland examination repeated daily during this period other diseases were eliminated and especially those due to *P. bigeminum*, *P. mutans*, and *Anaplasma marginale*. Three days after the commencing rise of temperature 3 to 5 litres (calves 1 litre) of blood were extracted and on the fourth day a similar quantity. If the animal was very much exhausted it was bled to death from the carotid. Animals which bore repeated blood extraction were only completely bled on the tenth day.

A month's collection of serum was titrated at the same time by injecting a series of cattle with 10, 20, 30, and 40 cc., respectively, of serum together with virus and the control animal with virus only. The serum was mixed with ½ per cent. carbolic acid and distributed in 250 cc. bottles. At this place 67,000 doses of serum were manufactured between January and August, 1913. The average dose consisted of 25 cc. of serum for a medium sized animal when used for the double inoculation.

As there are still districts, viz., the Urundi and Ruanda districts, which are free of rinderpest an endeavour was made to keep a large reserve of serum to cope with the possibility of outbreaks of rinderpest occurring in these districts.

The establishment of a second serum station was attended with some difficulties owing to the lack of suitable sites, but it was finally established in Mpapua and it commenced delivering serum in July, 1913. Wölfel was again sent to this station to superintend the manufacture of serum and also with the assistance of a trained staff to investigate some important problems, which are then detailed by the author, in connection with rinderpest.

Before the manufacture of serum had attained sufficient dimensions the ordinary prophylactic measures were adopted, consisting in prohibition of movement, destruction of dead bodies, and isolation of affected animals. Dung was removed from the isolation kraals but this was not possible everywhere as some of the natives (the Tagago) use these for burying their dead. Great difficulties were encountered in carrying out the policy of isolation. The flesh of the diseased cattle was allowed to be eaten provided it was well cooked, but the transport of raw flesh was forbidden on account of the danger of spreading the infection.

Koch's gall immunisation was also attempted on a fairly large scale. Though not very satisfactory it was found to be capable of giving fairly good results provided the gall was taken when fresh and from an animal which died seven or eight days after the commencement of the illness or was killed just before death. The immunity was prolonged if inoculation with virulent blood was performed 10 days after the gall inoculation. The production of abscesses at the point of inoculation gained disfavour for this method in the eyes of the natives. 10 cc. of gall was found necessary for immunising one bovine and as the gall bladder contains 500 cc. of gall a sick animal can furnish material sufficient to immunise 50

sick animals. The losses in the Bahi district amounted to 10 per cent and in Bugiri to 25 per cent. with this method.

The only means for the suppression and eradication of rinderpest in a country like German East Africa according to Ostertag is the simultaneous method, that is to say, inoculation with specific serum and virulent blood.

The objections raised against this method especially by the Bloemfontein Rinderpest Conference are referred to and on the other hand the short period of immunity conferred by serum alone, as shown by WARD's experiments in the Philippines, etc., is pointed out.

The experiments carried out at the Veterinary College in German East Africa demonstrated the superiority of this method. In East Africa the introduction of piroplasmosis with the virus was found to be unimportant; as nearly all the cattle are infected with these organisms they have all become immune. Also, rinderpest usually occurs in districts where the tsetse fly is unknown or seldom found and, moreover, the natives are very careful in guarding their cattle against the tsetse fly. The results of the simultaneous inoculation in German East Africa fulfilled all expectations. Failure only took place in two instances, viz., when an imported untitrated serum was issued and secondly when an infected serum was used. The losses as a rule amounted to only 1 per cent.

Instances are then given of outbreaks in which the beneficial results were easily observable. In the case of some animals transported to the coast for slaughter inoculation with serum alone was performed. As this transport in some cases took about six weeks or more Ostertag considers that the protection afforded was very doubtful.

Ostertag's conclusions are as follows:—

"(1) In all affected centres all animals from a milch calf up to an adult ox must be thoroughly inoculated in order to ensure safety. If this is not done dangerous rinderpest centres are created with regular sickening of the growing young cattle. The healthy animals must be treated by the simultaneous method and the affected ones with serum alone in double doses. If sufficient serum is not available gall inoculation can be resorted to with the above-described precautions. When inoculation is carried out in this way the insufficiently immunised animals die while the remainder are proof against rinderpest. Six weeks after the stamping out of the disease and after all dung has been burned the animals can be safely transported.

"(2) In addition to the diseased herds those in the neighbourhood should be inoculated and treated in the same way as animals in the affected districts. Calves may be inoculated with pure virus. As soon as sufficient serum is available the susceptible herds must be inoculated with serum only to prevent the spread of the disease.

"(3) For the safety of cattle in the settlers districts on the Meru and Kilimandjaro the entire herds should be innoculated.

In order to safeguard the supply of meat to the coast the same should be done to all cattle in the market districts.

"(4) If adequate serum is available all cattle on farms lying apart should be gradually inoculated to prevent a fresh outbreak. I also proposed that all skins should be sent to the nearest Government Station and there inspected by an official as to their complete dryness.

"If this is done the complete stamping out of the disease in German East Africa may be looked forward to."

Ostertag further recommended the inoculation of all cattle on the northern frontier owing to the danger of importing the disease from

the large native reserves in British East Africa. The danger from British East Africa should be guarded against on account of the permanence of rinderpest in Abyssinia and Somaliland.

ZONCHELLO (A.). **Il gulhai nel Sahel.** [Rinderpest in Sahel.]—*Clinica Vet.* 1917. Mar. 15. Vol. 30. No. 5. pp. 113–132.

The Sahel district is a long strip of country bordering the Red Sea in the Italian colony of Eritrea. Although rinderpest was very prevalent in other parts of the colony the Sahel district appears to have been free of disease until the year 1914, when after the return of a herd, which had been taken out of the district into the neighbourhood of an affected centre, in the rainy season rinderpest appeared and immediately caused a serious outbreak throughout Sahel. The mortality rose up to over 95 per cent. of the total head of cattle and caused great misery among the entirely pastoral population. Recourse was then made to a large extent to raising sheep and goats or to mixed flocks. In the following year rinderpest broke out afresh, and the disease has since become enzootic. The mortality, however, progressively decreased until it did not, except in rare instances, exceed 50 per cent. In rare cases the mortality was nil.

Rinderpest appears to have been first imported into Eritrea in 1887, and caused great havoc at the time. Its spread was occasioned, according to the author, through the incompetence and dilatoriness of the authorities in the administration of elementary prophylactic measures; instead, specific measures of treatment were attempted on a large scale, with, upon the whole, rather unsatisfactory results. In 1903 it was thus decided to adopt the simultaneous method, or KOLLE & TURNER'S method, just at the time when THEILER and the Bloemfontein Conference condemned it.

From inquiry among the natives the author then details the history of attempts at sero-vaccination in eight different outbreaks in Sahel from 1905 up to the time of his arrival (1914). In many instances, however, it appeared that although the results were quite satisfactory in the case of the animals treated the method created new centres of disease and a considerable spread from the original centre. The failure to get better results, however, judging from the figures presented by the author, appears to the reader to be due not to defects in the method, but to its not being rigorously or rationally carried out.

The British authorities in the Soudan protested against its use on the Anglo-Italian frontier in the winter 1914–1915 as the Italians were accused of maintaining the virus and distributing it.

The method adopted by the author on his arrival, after a study of the disease, was to proceed simply by strict isolation of existing centres. The results obtained in less than a year were considered to be most satisfactory and it is believed that if this plan was adopted throughout the colony and a close watch kept on the Abyssinian frontier rinderpest would soon be caused to disappear almost entirely. This method seems also to be more in favour among the natives, who soon learn to recognise its advantages.

It is believed that wild animals are susceptible to rinderpest and sero-vaccination should be particularly forbidden where these exist. The affected wild animals are said to isolate themselves from other

animals. According to wide enquiry among the natives goats do not naturally contract rinderpest in this district, and, moreover, they believe that cattle, such as transport cattle living among herds of goats, do not contract the disease in the scene of an outbreak. The author quotes an observation of his own which corroborates the view of the natives.

Unnecessary delay is caused in treating a herd by inoculation. It is held by many people in the district that the Serum Institute at Asmara prevents the disease from being finally extinguished in the colony. The cost of sero-vaccination, moreover, amounts to about 25 per cent. of the value of the inoculated animals.

(c) Nairobi Sheep Disease.

Montgomery (Eustace). **On a Tick-borne Gastro-Enteritis of Sheep and Goats occurring in British East Africa.**—*Jl. Comp. Path. and Therap.* 1917. Mar. Vol. 30. Pt. 1. pp. 28–57. With 6 charts.

A brief reference to this condition was made in the Annual Report of the Veterinary Bacteriologist for East Africa for the year 1914-1915 [see this *Bulletin*, Vol. 5, No. 1, p. 66]; the experiments upon which the conclusions were based are detailed in this paper.

Field observations. The disease was first brought under the author's notice in 1910 when heavy mortality was observed among the large numbers of sheep brought by traders from various outlying areas into the Nairobi district. The epizootiology of the disease up to 1915 is then briefly discussed. It is considered that the disease affects essentially the Kikuyu country, which extends from Mount Kenia to Nairobi. No immunity was ever discoverable in sheep in the outlying districts, such as the Masai Reserve, from which sheep were usually imported, and these districts are probably free from infection; the brown tick, *Rhipicephalus appendiculatus*, is remarkably rare in these districts and this probably accounts for their freedom from this disease and East Coast fever.

The mortality among native sheep in naturally occurring outbreaks appears to be very high; in the first observed outbreak 2,000 sheep died out of 3,000 within one month of arrival in the infected area.

The symptoms are briefly those of dulness to commence with, followed shortly afterwards by severe diarrhoea and abdominal pain. Death usually occurs within two days after the onset of symptoms. The temperature is normal while visible symptoms are exhibited, but a characteristic curve is manifested for a day or two before these are observed.

On post-mortem examination one notices evidence of diarrhoea, often a blood-stained discharge from the nostrils, and on examination of the internal organs one finds an acute, often haemorrhagic, inflammation of the mucous membrane of the fourth stomach and of the intestines, splenic enlargement, distension of the gall bladder, and congestion of the trachea.

Laboratory observations. When susceptible sheep have been inoculated with virulent blood a sudden rise of temperature up to 105° or 106° F. is usually observed within 48 hours. This remains high—between 105° and 108° F.—and then falls suddenly to normal, where it remains for one or two days before death or recovery takes place.

There appears to be some difference in the virulence of various strains, the minimum period between inoculation and the initial rise of temperature varying from 36 to 84 hours. Prior to death the temperature may be subnormal. Death usually occurs between four and eight days after inoculation—average 148 hours.

Following infection with ticks the incubation period averaged 9·4 days—minimum 5 and maximum 16—and the reaction 3·6 days.

No visible symptoms could be noted until about the last day of the reaction when symptoms set in as in the natural cases commencing with dulness, loss of appetite, diarhoea, mucous discharge from the nostrils, etc. In the rare cases of recovery the symptoms were usually limited to those of dulness, inertia, and slight anaemia. The onset of diarrhoea appears invariably to indicate a fatal termination. Out of a total of 224 sheep inoculated 3 only failed to react and 130 died. The mortality was much heavier among native sheep (71·5 per cent.) than in half-bred (31·5 per cent.), or in Merino sheep (30·7 per cent).

The post-mortem appearances of animals dead from natural infection from experimental infection with ticks or by inoculation are very constant; these are described in detail by the author.

Sheep and goats appear to be the only animals susceptible. Out of 20 goats, mostly half-bred Angora, one only proved to be immune on inoculation; three gave no temperature reaction but the virus was shown to exist in two of these by sub-inoculation; five gave a very slight temperature reaction but showed no symptoms. The blood, however, was shown to be virulent on inoculation. The remaining 11 goats reacted as in the case of the sheep, but the onset of the disease was delayed by about a day and the symptoms were much milder. A transitory diarrhoea was noted in five only. Two goats died of gastro-enteritis, one on the 6th day and one on the 11th day.

Attempts were made to infect cattle, buffalo, horses, mules, donkeys, and other animals, but negative results only were obtained and their blood was found to be avirulent on sub-inoculation, with one exception in which the blood of an ox was found to be infective 24 hours after intravenous inoculation of a large dose of virus.

Experiments to determine the virulence of the various body fluids and tissues showed that the blood was always virulent when taken during the thermal reaction. It was found that the virus was not destroyed by short contact with equal parts of oxalate-carbol-glycerine solution (oxalate of potash 5 grammes, carbolic acid 5 grammes, glycerine 1,000 c.c. and water 1,000 c.c.). The virus was found to be present in the blood for about one day subsequent to the return to normal temperature. Serum obtained from blood during the infective period was proved to be virulent both on direct inoculation and after filtration. Saline extracts of organs removed from animals dying with the acute reaction were virulent. When the temperature had been normal for some days and the clinical symptoms were present, negative results were frequently obtained. The pericardial fluid obtained from a sheep dead from the acute form of the disease was infective.

The bile gave negative results.

Urine obtained from the bladder of a sheep after death was found to be infective when given in a dose of 10 c.c. subcutaneously. Urine

obtained during life at the height of a reaction gave positive results in two cases on inoculation while six experiments were negative, but in two of the latter cases the urine was administered *per os*.

Out of five sheep drenched with faeces obtained after death from animals dead of the disease three did not react while the remaining two died on the 7th and 18th days, respectively, without manifesting any temperature reaction and subinoculations made from one were negative. A watery decoction of faeces passed through a Berkefeld filter failed to infect on subcutaneous inoculation.

A study of the various methods of experimental infection showed that the disease could be regularly and constantly transmitted by subcutaneous inoculation as well as by intravenous and intraperitoneal inoculation. The disease was also readily transmitted by intracutaneous inoculation performed by applying a drop of citrated virulent blood to the shaved and lightly scarified ears of two sheep. The minimum lethal dose of serum was shown to be as low as 0·001 c.c. in one test.

When administered *per os* virulent blood was found to be infective in three cases in doses of 50 c.c. Ten c.c., however, failed to produce a reaction. Two sheep fed with dry lucerne over which 100 c.c. had been sprinkled reacted and died. Another sheep fed with grass over which 50 c.c. of urine obtained after death had been sprinkled remained unaffected. The same sheep when fed with grass over which 50 c.c. of virulent blood had been sprinkled also remained healthy, but when later fed with grass on which 50 c.c. of virulent blood had been sprinkled a reaction occurred and death took place on the 9th day.

Experiments showed that it was not possible to transmit the disease by intimate contact in pens or in a small paddock. It was proved that the natural method of transmission was through the agency of ticks—*Rhipicephalus appendiculatus*—which had fed as nymphae on reacting sheep.

Bacteriology. Direct microscopic examination was negative and no organism was cultivable on ordinary media.

The filterability of the virus was demonstrated in numerous experiments, in which blood, in dilutions of from 1 to 4 up to 1 to 999 with saline solution or with tap water, was passed through a Berkefeld No. 7 and Chamberland F candles. The number of negative results formed a small minority and these were probably due to the use of occluded candles.

Experiments to ascertain the resistance of the virus showed that a recovered animal is not a reservoir for the virus. Blood taken from recovered animals whose temperature had been normal for from 7 to 10 days failed to infect even in large doses. The resistance of the virus *in vitro* was found to be comparatively long. Sterile serum or citrated blood was found to retain its virulence for about 28 days when exposed to the air as in plugged flasks, for up to 45 days when contained in sealed tubes or ampoules, and for about a week when mixed with equal parts of oxalate-carbol-glycerine solution.

Citrated blood kept at 37° C. in the incubator for 42 hours retained its virulence. When dried *in vacuo* at 37° C. for 72 hours the virulence had disappeared.

A temperature of 50° C. acting for one hour did not destroy the virus; exposure for two hours at this temperature produced partial

destruction, the inoculated sheep developing only a temperature reaction and becoming immune to subsequent inoculation. Inoculation of a fairly large number of sheep with blood thus exposed for 1½ hours produced variable results; about a half developed a delayed reaction and died while nearly all the rest showed slight reactions and became immune.

A temperature of 60° C. acting for from 5 minutes to 1½ hours completely destroyed the virulence.

Mode of transmission. A number of experiments are detailed in which susceptible sheep were grazed over pastures believed to be contaminated by the infection, "and on every occasion one or more contracted the disease even if they were so muzzled as to prevent the animal from eating the grass or drinking the water of that infected area."

Sheep fed on grass cut from an infected area did not contract the disease, but it is remarked that the grass obtainable in these cases was procured from the longer growths occurring close to the village huts and neglected by sheep, and not the short turfy couch grass grazed over and harbouring ticks.

The rôle of ticks in the transmission of the disease was ascertained in the first place in a number of experiments with ticks obtained from naturally reacting sheep. A number of adult *Rhipicephalus appendiculatus* and a few adult *Amblyomma variegatum* emerged from the nymphae. Those obtained from all the other stages of ticks collected died prematurely. When applied to susceptible sheep *R. appendiculatus* was found invariably to transmit the infection while the other species of tick gave negative results.

Numerous experiments were also performed with laboratory-bred ticks. It was found impossible to convey the disease by means of larval *R. appendiculatus* born of mothers which had fed on reacting sheep. Likewise nymphae which were born of mothers feeding on sick animals and which did not convey the disease in the larval stage failed to convey the infection. Ticks which had fed as larvae on reacting sheep and failed to transmit the infection as nymphae also failed to convey the disease in the adult stage.

Experiments, however, showed that ticks which had fed as nymphae on reacting sheep in most cases transmit the disease as adults. The blood of animals thus infected was found to be virulent on sub-inoculation.

Immunity. An animal which has recovered from the natural or the experimental disease does not react to a second infection and tests indicated that the duration of this immunity may extend for several years. Half-bred and pure-bred sheep appear to be much more resistent to infection than native sheep. Owing to the peculiar conditions of grazing adopted by the natives the disease appears to be very localised even in infected areas.

Artificial immunisation yielded by no means promising results. Attempts were made to prepare an anti-serum by inoculating immune sheep subcutaneously, intra-peritoneally and intramuscularly with citrated virulent blood in doses up to 500 c.c. This dose could be given as a second injection. After a number of injections the animals were bled in about a fortnight after the last injection and a further maximum injection was made if necessary.

"The sera obtained from each animal, or from a series of animals, after each hyperimmunising were kept separate and used in independent tests, either alone or in association with virus given simultaneously or at variable intervals later.

"No clearly satisfactory results were obtained. It appeared that when sheep were strongly hyperimmunised the sera acquired haemolytic properties and killed a higher proportion of animals than it saved; and if fortification was not intense it possessed little or no prophylactic powers.

"As the object of this work was to devise measures which would safeguard the sheep-owner should the disease gain entrance to his flocks, it became apparent that serum prophylaxis, entailing the manufacture of expensive serum, would not be a practicable method except in extreme cases. Further, additional research indicated that the disease did not exist in the districts wherein sheep farming was extensively carried on, and as this line of preventive treatment could not yet be applied to the native Reserves the enquiry was suspended."

A study of the phenomena of haemolysis in sheep inoculated with the serum, together with details of results obtained, next follow.

A small number of experiments indicated that a quite appreciable attenuation of the virus is effected by passage through goats, and that the results appear to justify observation on a more extended scale.

Prevention. Movement of affected flocks should be prohibited, but this is difficult to effect in practice.

"Individual owners of land where *Rhipicephalus appendiculatus* exists should not permit the entry of doubtful sheep until they have undergone a quarantine of at least 21 days without any deaths occurring from this disease. The sheep might then be introduced. Should deaths occur, the quarantine area grazed over must be regarded as infected; and, though the sheep can then be moved without fear, it should be destocked of sheep and goats for eighteen months, and grazed with cattle only for that period."

A small experiment indicated that the dipping of sheep at three-day intervals might be considered beneficial.

"The eradication of ticks capable of carrying the disease should form the basis of preventive measures.

"*Rhipicephalus appendiculatus* is also the carrier of East Coast fever in cattle, and for its eradication cattle must be dipped every three days.

"There is no doubt that dipping cattle at a three-day interval will very greatly clear the ground of all ticks, and in their absence gastro-enteritis of sheep cannot spread."

(d) Variola.

Gauducheau (A.). **Recherches sur la variole-vaccine.** [Investigations on Variola-Vaccinia.]—*Bull. Soc. Path. Exot.* 1917. Mar. Vol. 10. No. 3. pp. 260–268.

In this memoir the author presents the results of experiments made during the course of the last ten years at the Vaccine Institute, Tonkin (French Indo-China), on the relationships between variola and vaccinia.

The following are his conclusions:—

"(1). There exists no symptom by which one can absolutely and constantly differentiate between variola and vaccinia.

"(2). Variola immunises against vaccinia and vice versa.

"(3). A few attempts at the transformation of variola into vaccinia have given positive results.

"(4). The differences which exist between the two states of the virus appear to be solely in the degree of adaptation to the susceptible species and constitute two varieties or types of the same virus: the human type or variola and the bovine type or vaccinia."

MISCELLANEOUS.

VAN SACEGHEM (R.). **Etude de tumeurs constatées sur une génisse de la race zebu.** [Tumours in a Heifer of the Zebu Breed.]—*Bull. Soc. Path. Exot.* 1917. Mar. Vol. 10. No. 3. pp. 182–183.

A zebu heifer kept at Zambi showed over a period of two years a large number of warty growths around the anus. These growths spread around the vulva and over the tail and then affected the teats, limbs, and the right auditory meatus. The last-named localisation brought about the death of the animal. The tumours were found to be contagious for the animal itself. The warts were composed of a fibrous base covered with a cluster of small, very fragile, reddish, transparent papillomata from 5 mm. to 1 cm. in length by a few mm. in breadth.

These papillomata are often found as complications of tropical dermatitis in bovines [see above, extract p. 108].

A description is given of the histology of the lesions.

RECENT LITERATURE.

[Continued from this *Bulletin*, Vol. 5, No. 1, pp. 77–84.]

PROTOZOAN PARASITES.

Alexeieff (A.). Mitochondries et corps parabasal chez les Flagellés. [Mitochondria and Parabasal Bodies in Flagellates.]—*C.R. Soc. Biol.*, 1917. Mar. 31. Vol. 80. No. 7. pp. 358–361. With 1 fig.

——. Mitochondries et rôle morphogène du noyau. [Mitochondria and Morphogenetic Rôle of the Nucleus.]—*Ibid.* pp. 361–363.

Behrend (Kurt). Zur Conjugation von Loxocephalus. [Copulation in Loxocephalus.]—*Arch. f. Protistenk.*, 1916. Aug. Vol. 37. No. 1. pp. 1–5. With 1 coloured plate.

Breuer (Rudolf). Fortpflanzung und biologische Erscheinungen einer *Chlamydophrys*-Form auf Agarkulturen. [The Growth and Biological Phenomena of a Chlamophrys Organism on Agar.]—*Arch. f. Protistenk.*, 1916. Aug. Vol. 37. No. 1. pp. 65–92. With 2 text figs. & 1 plate.

Comadon (J.). Phagocytose in vitro des Hématozoaires du Calfat (enrégistrement cinematographique). [Phagocytosis *in vitro* of the Haematozoa of the Java Sparrow. (Cinematographic representation.]—*C.R. Soc. Biol.*, 1917. Mar. 17. Vol. 80. No. 6. pp. 314–316. With 1 plate comprising 2 figs.

Léger (L.) & Duboscq (O.). Sporozoaires de *Glossobalanus minutus* Kow. *Eimeria epidermica* n. sp.; *Eimeria beauchampi* n. sp.; *Selenidium metchnikovi* n. sp.—*Ann. Inst. Pasteur*, 1917. Feb. Vol. 31. No. 2. pp. 60–72. With 3 plates.

—— & Hesse (E.). Sur les Microsporidies de la crevette d'eau douce. [The Microsporidia of the Fresh-Water Prawn.]—*C.R. Soc. Biol.*, 1917. Jan. 6. Vol. 80. No. 1. pp. 12–15. With 6 figs.

—— & Hollande (A. C.). Sur un nouveau protiste à facies de *Chytridiopsis*, parasite des ovules de l'huitre. [A New Protozoon resembling Chytridiopsis, a Parasite of the Ovules of the Oyster.]—*C.R. Soc. Biol.*, 1917. Jan. 20. Vol. 80. No. 2. pp. 61–64. With 4 text figs.

Keilin (D.). Une nouvelle entamibe, *Entamoeba mesnili* n. sp., parasite intestinal d'une larve d'un diptère. [A New Entamoeba, *E. m.*, an Intestinal Parasite of a Dipterous Larva.]—*C.R. Soc. Biol.*, 1917. Feb. 3. Vol. 80. No. 3. pp. 133–136. With 25 figs.

Pascher (A.). Rhizopodialnetze als Fangvorrichtung bei einer plasmodialen Chrysomonade. [Trap Apparatus in the Form of Rhizopodial Threads in a Plasmodial Chrysomonad.]—*Arch. f. Protistenk.*, 1916. Aug. Vol. 37. No. 1. pp. 15–30. With 6 text figs. & 1 coloured plate.

——. Fusionsplasmodien bei Flagellaten und ihre Bedeutung für die Ableitung der Rhizopoden von den Flagellaten. [Fusion Plasmodia in Flagellates and their Significance regarding the Origin of Rhizopoda in Flagellates.]—*Ibid.* pp. 31–64. With 20 text figs. & 1 coloured plate.

Poche (Franz). Die Verwandtschaftsbeziehungen der vermeintlichen Gregarine *Microtaeniella clymenellae* Calk. [Relationships of the supposed Gregarine *M. c.*]—*Arch. f. Protistenk.*, 1916. Aug. Vol. 37. No. 1. pp. 6–14.

Sangiorgi (Giuseppe). Sulla cultura in vitro degli spironemi dell' intestino umano. [Cultivation in vitro of Spirochaetes from the Human Intestine.]—*Pathologica*, 1917. Feb. 15. Vol. 9. No. 198. pp. 61–62.

Shortt (H. E.). Notes on Two Haemogregarines of Cold-Blooded Vertebrates.—*Indian Jl. Med. Res.*, 1917. Jan. Vol. 4. No. 3. pp. 402–413. With 2 coloured plates.

Swellengrebel (N. H.) & Schiess (J. R.). Quelques remarques sur la morphologie de l' *Entamoeba histolytica* et la valeur diagnostique de l'infection rectale des chats. [Some Remarks on the Morphology of *E.h.* and the Diagnostic Value of Rectal Inoculation of Cats.]—*Bull. Soc. Path. Exot.*, 1917. Jan. Vol. 10. No. 1. pp. 13–17. With 4 text figs.

Watson (Minnie E.). Observations on Polycystid Gregarines from Arthropoda.—*Jl. Parasit.*, 1916. Dec. Vol. 3. No. 2. pp. 65–75. With 1 plate.

METAZOAN PARASITES.

Arthropods (Acari, Flies, Ticks).

Burkhardt (F.). Die Larve des Speckkäfers (*Dermestes lardarius*, L.), ein Feind des schlüpfenden Geflügels. [The Larva of the Bacon Beetle, an Enemy of Hatching Poultry.]—*Berlin. Tier. Woch.*, 1917. Jan. 25. Vol. 33. No. 4. pp. 44–45.

Carpano (M.). Sulla diagnosi clinica e microscopica delle piu frequenti dermatosi parassitarie degli equini. [Clinical and Microscopic Diagnoses of the more Common Skin Affections in the Horse.]—*Moderno Zooiatro*, 1916. Nov. 30 & Dec. 31, & 1917, Jan. 31. Vol. 5 & 6. Nos. 11, 12 & 1 respectively. pp. 261–277, 289–302, & 7–17. With 13 figs.

Hadwen (S.). A further Contribution on the Biology of *Hypoderma Lineatum*.—*Canada Dept. of Agric. Health of Animals Branch. Scientific Series.—Bull.* No. 21. 1916. Ottawa: Govt. Printing Bureau.

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Henry. Otacariases et prophylaxie des gales psoroptiques. [Otacariases and Prophylaxis of Psoroptic Mange.]—*Bull. Soc. Cent. Méd. Vét.*, 1917. Jan. 4.—*Rec. Méd. Vét.*, 1917. Jan. 30–Feb. 28. Vol. 92. Nos. 3 & 4. pp. 41–48.

Howard (L. O.). Report of the Entomologist.—*U.S. Dept. Agric., Bur. of Entom., Washington, D.C.*, 1916. Aug. 24. pp. 8–11.

v. Kemnitz (G. A.). Untersuchungen über den Stoffbestand und Stoffwechsel der Larven von *Gastrophilus equi* (Clark), nebst Bemerkungen über den Stoffbestand der Larven von *Chironomus* (spec?) L. (Physiologischer Teil). [Investigations on the Nature of and Changes in the Composition of the Larva of *Gastrophilus equi* with Remarks on the Nature of the Larva of *Chironomus* sp.]—*Zeitschr. f. Biologie*, 1916. Dec. 16. Vol. 67. Nos. 3, 4 & 5. pp. 129–244. With 2 plates and 5 text figs.

Mitzmain (M. B.). A Digest of the Insect Transmission of Disease in the Orient with Especial Reference to the Experimental Conveyance of *Trypanosoma evansi*.—*New Orleans Med. & Surg. Jl.*, 1916. Dec. Vol. 69. No. 6. pp. 416–424.

[This is for the most part a résumé of a previous paper that was pretty fully extracted in the preceding number of this *Bulletin*.]

Quiros (D.). Biologia de la Nigua [Biology of the Chigger.]—*Anales Hosp. de San José*, 1916. Nov. 1. Vol. 2. No. 1. pp. 1–17. With 4 figs.

Sweet (Georgina) & Seddon (H. R.). The Viability of *Melophagus ovinus*, Linn., the Sheep Louse-Fly, Sheep Ked, or Sheep "Tick." *Vet. Jl.*, 1917. Apr. Vol. 73. No. 4. Australian Supplement. pp. 6–14. With 3 tables.

Taylor (Frank H.). *Sarcophaga froggatti*, sp. n. A New Sheep-Maggot Fly.—*Bull. Entom. Res.*, 1917. Jan. Vol. 7. No. 3. p. 265.

du Toit. Ueber das Sammeln und die Zucht unserer heimischen Zecke *Ixodes ricinus* L. [Collection and Breeding of the German Tick *I.r.*]—*Berlin. Tier. Woch.*, 1917. Mar. 8 & 15. Vol. 33. Nos. 10 & 11. pp. 109–112 & 124–127. With 4 figs.

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Waterston (James). Notes on the Morphology of Chalcidoidea bred from Calliphora.—*Parasitology*, 1917. Feb. Vol. 9. No. 2. pp. 190–198. With 2 text figs.

van Zwaluwenburg (R. H.). Report of the Entomologist.—*Rept. Porto Rico Agric. Expt. Sta.*, 1915. 1916. Nov. 23. Washington D.C. p. 42.

["The cattle tick (*Margaropus annulatus australis*) is being studied with a view to working out a method of extermination by pasture rotation applicable to Porto Rico. This tick is undoubtedly a most serious drain on the island's prosperity, but it is hoped that its ultimate extermination is possible."—*Rev. App. Entom.* Series B. 1917. Apr. Vol. 5. Part 4. p. 64.]

Helminths.

Bauer. Untersuchungen über Sommerwunden. [Researches on Summer Sores.]—*Zeitsch. f. Veterinärk*, 1917. No. 1. p. 1. (Extracted in *Berlin. Tier. Woch.*, 1917. Mar. 1. Vol. 33. No. 9. p. 103).

Boulenger (Charles L.). Sclerostome Parasites of the Horse in England. II. New Species of the Genus *Cylichnostomum*.—*Parasitology*. 1917. Feb. Vol. 9. No. 2. pp. 203–212. With 5 text figs.

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